Report on the Deliberation Results

February 15, 2013
Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau,
Ministry of Health, Labour and Welfare

[Brand name]	Acofide Tablets 100 mg
[Non-proprietary name]	Acotiamide Hydrochloride Hydrate (JAN*)
[Applicant]	Zeria Pharmaceutical Co., Ltd.
[Date of application]	September 29, 2010

[Results of deliberation]
In the meeting held on February 7, 2013, the First Committee on New Drugs concluded that the product may be approved and that this result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council.

The product is not classified as a biological product or a specified biological product, the re-examination period is 8 years, and neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug.

**Japanese Accepted Name (modified INN)*

Review Report

January 25, 2013
Pharmaceuticals and Medical Devices Agency

The results of a regulatory review conducted by the Pharmaceuticals and Medical Devices Agency on the following pharmaceutical product submitted for registration are as follows.

[Brand name]	Acofide Tablets 100 mg
[Non-proprietary name]	Acotiamide Hydrochloride Hydrate
[Applicant]	Zeria Pharmaceutical Co., Ltd.
[Date of application]	September 29, 2010
[Dosage form/Strength]	Each tablet, containing 100 mg Acotiamide Hydrochloride Hydrate
[Application classification]	Prescription drug (1) Drug with a new active ingredient

[Chemical structure]

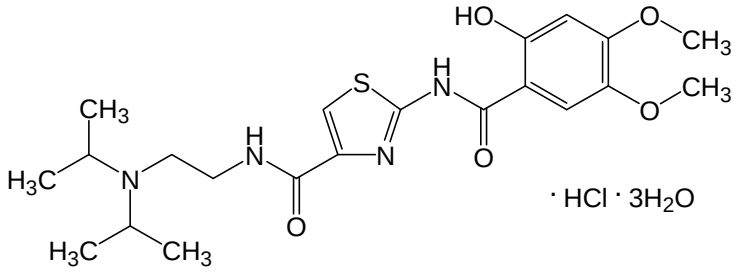
Molecular formula $C_{21}H_{30}N_4O_5S \cdot HCl \cdot 3H_2O$

Molecular weight 541.06

Chemical name

N-{2-[Bis(1-methylethyl)amino]ethyl}-2-[(2-hydroxy-4,5-dimethoxybenzoyl) amino]thiazole-4-carboxamide monohydrochloride trihydrate

Structural formula



[Items warranting special mention]	None
[Reviewing office]	Office of New Drug I

Review Results

January 25, 2013

[Brand name]	Acofide Tablets 100 mg
[Non-proprietary name]	Acotiamide Hydrochloride Hydrate
[Applicant]	Zeria Pharmaceutical Co., Ltd.
[Date of application]	September 29, 2010

[Results of review]

Based on the submitted data, it is concluded that the efficacy of the product in treatment of postprandial fullness, upper abdominal bloating, and early satiation in patients with functional dyspepsia has been demonstrated and its safety is acceptable.

As a result of its regulatory review, the Pharmaceuticals and Medical Devices Agency has concluded that the product may be approved for the following indication and dosage and administration.

[Indication]	Postprandial fullness, upper abdominal bloating, and early satiation in patients with functional dyspepsia
[Dosage and administration]	The usual adult dosage is 100 mg of acotiamide hydrochloride hydrate administered orally three times daily before a meal.

Review Report (1)

December 10, 2012

I. Product Submitted for Registration

[Brand name]	Acofide Tablets 100 mg
[Non-proprietary name]	Acotiamide Hydrochloride Hydrate
[Applicant]	Zeria Pharmaceutical Co., Ltd.
[Date of application]	September 29, 2010
[Dosage form/Strength]	Each tablet, containing 100 mg Acotiamide Hydrochloride Hydrate
[Proposed indication]	Functional dyspepsia (gastrointestinal symptoms such as postprandial fullness, upper abdominal bloating, and early satiation)
[Proposed dosage and administration]	The usual adult dosage is 100 mg of acotiamide hydrochloride hydrate administered orally three times daily before a meal.

II. Summary of the Submitted Data and Outline of the Review by the Pharmaceuticals and Medical Devices Agency

A summary of the submitted data and an outline of the review by the Pharmaceuticals and Medical Devices Agency (PMDA) are as shown below.

1. Origin or history of discovery and usage conditions in foreign countries etc.

Functional dyspepsia (FD) is defined as conditions in which upper abdominal symptoms are present in the absence of any organic disease that may explain the symptoms. According to the Rome III criteria, the international diagnostic criteria, FD is defined as "a disease that started at least 6 months prior to diagnosis, showing at least one of the following symptoms for the last 3 months: bothersome postprandial fullness, early satiation, epigastric pain, epigastric burning; and there is no evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms."

The cause of FD is not fully elucidated. The current assumption is that the symptoms are caused by the involvement of multiple factors including abnormal gastric emptying, gastric dysrhythmia, gastric hypersensitivity, hypersensitivity and motility disorder of the small intestine, impaired postprandial relaxation of the gastric fundus, vagus nerve disorder, enhanced acid sensitivity, psychological factors, and central nerve disorders (*Frontiers in Gastroenterology*. 2007;12:8-30). Although not a fatal disease, FD is considered to significantly affect the quality of life (QOL) of patients (*Am J Gastroenterol.* 2009;104:1483-1488, *Aliment Pharmacol Ther.* 2008;27:1148-1155), and the symptoms, even if they have improved, may relapse repeatedly, resulting in a prolonged period of suffering (*Gastroenterology*. 1987;92:1060-1066).

In Japan, FD is currently treated with histamine H_2 receptor blockers, proton pump inhibitors, and prokinetic agents alone or in combination, depending on the symptoms of the patient. However, there are no reports that have the demonstrated distinct efficacy of these drugs in patients with FD that fulfills the Rome criteria.

Acotiamide hydrochloride hydrate (hereinafter referred to as "acotiamide") is an acetylcholinesterase (AChE) inhibitor discovered by the applicant. Acotiamide suppresses the degradation of acetylcholine (ACh) released from cholinergic nerve terminals by inhibiting AChE, thereby enhancing the ACh-induced contraction and motility of the gastric antrum and the gastric body. Therefore, on the basis of the expectation that acotiamide would enhance the

movement of the gastric antrum and improve the gastric hypomotility in patients with FD, development of the drug was initiated.
Acotiamide has not been approved in any foreign country as of November 2012.

2. Data relating to quality

2.A Summary of the submitted data

2.A. (1) Drug substance

2.A.(1).1) Characterization

The drug substance is white to pale yellow crystals or crystalline powder, and has been determined for description, solubility, hygroscopicity, melting point, pH, dissociation constant (pKa), distribution coefficient, thermal characteristic (TG-DTA curve), and crystalline polymorphism. The drug substance is a trihydrate and has a single crystal form. No crystalline polymorphism is observed.

The chemical structure of the drug substance has been elucidated by elementary analysis, ultraviolet spectroscopy, infrared spectrophotometry (IR), water content, hydrochloric acid content, nuclear magnetic resonance spectrometry (^{1}H-, ^{13}C-NMR), mass spectrometry, and X-ray crystallography.

2.A.(1).2) Manufacturing process

The drug substance is manufactured using [redacted], [redacted], and [redacted] as the starting materials through [redacted] processes and [redacted] processes. The processes for manufacturing [redacted] and [redacted] are defined as the critical processes. Since the final intermediate [redacted] may directly affect the quality of acotiamide hydrochloride hydrate, it is controlled as the critical intermediate.

2.A.(1).3) Control of drug substance

The proposed specifications for the drug substance include content, description (appearance), identification (IR, qualitative test for chloride), purity (heavy metals, related substances [high performance liquid chromatography (HPLC)], residual solvents [gas chromatography]), water content, residue on ignition, thermal analysis, and assay (HPLC).

2.A.(1).4) Stability of drug substance

Table 1 shows the results of stability tests of the drug substance. Results of the photostability test showed that the drug substance was stable with light.

Table 1. Stability tests of drug substance

Test	Primary batches	Temperature	Humidity	Storage configuration	Storage period
Long-term stability testing	4 pilot batches	30°C ± 2°C	65% RH ± 5% RH	Polyethylene bags	60 months
Accelerated testing		40°C ± 2°C	75% RH ± 5% RH		6 months

Based on the above results, a re-test period of 60 months has been proposed for the drug substance when stored at room temperature in low-density polyethylene bags.

2.A.(2) Drug product

2.A.(2).1) Drug product and formulation

The drug product is white film-coated tablets, each containing 100 mg of the drug substance

acotiamide hydrochloride hydrate. It also contains, as excipients, lactose hydrate, microcrystalline cellulose, low substituted hydroxypropylcellulose, hydroxypropylcellulose, light anhydrous silicic acid, magnesium stearate, hypromellose, titanium oxide, and carnauba wax.

2.A.(2).2) Manufacturing process

The drug product is manufactured by a process comprising [redacted], [redacted], mixing, tableting, coating, and packaging and labeling, of which [redacted], [redacted], and [redacted] processes are regarded as critical processes.

2.A.(2).3) Control of drug product

The proposed specifications for the drug product include content, description (appearance), identification (IR), uniformity of dosage units (content uniformity [ultraviolet-visible spectrophotometry]), dissolution (ultraviolet-visible spectrophotometry), and assay (HPLC).

2.A.(2).4) Stability of drug product

Table 2 shows the results of the stability tests of the drug product. Results of the photostability test showed that the drug product was stable with light.

Table 2. Stability test of drug product

Test	Primary batches	Temperature	Humidity	Storage configuration	Storage period
Long-term stability testing	3 batches manufactured in a planned commercial scale[a)]	30°C	65% RH	PTP packaging, Polyethylene bottle packaging	36 months
Accelerated testing		40°C	75% RH		6 months

a) Planned commercial scale: [redacted] tablets, commercial scale: [redacted] tablets

Based on the above results, the shelf life of 36 months has been proposed for the drug product when stored at room temperature in PTP sheet packaging or in polyethylene bottle packaging.

2.B. Outline of the review by PMDA

2.B. (1) Description (appearance) of drug substance

The description of the drug substance is "white to pale yellowish white crystals or crystalline powder," and the drug substance manufactured by the previous manufacturing process had color, such as pale yellowish white, whereas all batches of the drug substances manufactured according to the current manufactures were white. PMDA therefore asked the applicant to set the specifications based on the results obtained from the current manufacturing process.

The applicant responded as follows:

It has been known that, even by the current manufacturing process, acotiamide hydrochloride hydrate is colored due to the color of the starting materials and the manufacturing process of crude acotiamide hydrochloride hydrate. Therefore, some of the future batches may also be colored. The drug substance with pale yellowish white color was also used in the evaluation in the nonclinical and clinical studies and the safety of such product has been confirmed. Furthermore, since the quality attributes of this drug substance, other than the description, are comparable with those of the drug substance manufactured at a pilot scale according to the current manufacturing process, the difference of the color (white vs. white to pale yellowish white) does not significantly affect the quality attributes of the drug substance other than the appearance. In addition, it has been confirmed that the pale yellowish white color of the drug substance does not affect the appearance of the drug product.

On the basis of the above, the applicant considers that there is no problem to specify the color of the drug substance as white to pale yellowish white.

PMDA considers as follows:
It is important to ensure the consistency of the quality by more appropriately implementing the manufacturing controls proposed at the regulatory submission. However, given that the drug substance with a pale yellowish white color was used in nonclinical and clinical studies and no particular safety problems were noted, it is acceptable to specify the color as "white to pale yellowish white."

Also, after reviewing the submitted data, PMDA asked the applicant to clarify ambiguous points and correct inappropriate descriptions mainly regarding the manufacturing process and specifications. The applicant responded to the instructions accordingly, and PMDA accepted the response of the applicant.

3. Non-clinical data

3.(i) Summary of pharmacology studies

3.(i).A. Summary of the submitted data

The following primary pharmacodynamic studies were conducted: studies on the gastrointestinal motility, on gastric emptying, and on their mechanisms. In the secondary pharmacodynamic study, the effect on basal gastric juice secretion was investigated. For safety pharmacology studies, effects on the central nervous system, cardiovascular system, respiratory system, etc., were investigated in GLP compliance studies (except in some studies). As a pharmacodynamic interaction study, interactions with acid secretion inhibitors were investigated.

Male animals were used in *in vivo* studies unless stated otherwise. In all studies, the dose of each study drug containing acotiamide hydrochloride hydrate (hereafter referred to as "acotiamide") was expressed in the amount of the salt, while the plasma concentration of acotiamide hydrochloride hydrate was expressed in that of acotiamide.

3.(i).A.(1) Primary pharmacodynamics

3.(i).A.(1).1) Effect on gastrointestinal motility

(a) Effect on the postprandial antral motility in dogs (4.2.1.1-21: Study 611[redacted]035)

A single oral dose[1] of the vehicle (empty capsule), acotiamide (3, 10, 30 mg/kg), itopride hydrochloride (itopride, 30 mg/kg), or mosapride citrate hydrate (mosapride, 10 mg/kg) was administered orally to dogs with a strain gauge force transducer (SGT) sutured onto the gastric antrum and, after 30 minutes, the dogs were fed. Table 3 shows the integrated value of the motion waveform every 2 hours (motility index) calculated from that up to 4 hours after feeding. Acotiamide significantly enhanced the postprandial antral motility at ≥10 mg/kg. Similarly, itopride and mosapride significantly enhanced the postprandial antral motility.

[1] Acotiamide, itopride, or mosapride at each dose was administered to the same animal after a washout period of at least 5 days. The vehicle (empty capsule) was administered 1 to 2 days before each administration.

Table 3. Effect on the postprandial antral motility in dogs

Treatment group	Motility index (g·min)		Treatment group	Motility index (g·min)	
	0-2 h	2-4 h		0-2 h	2-4 h
Control group (vehicle)	796.0 ± 96.8	748.0 ± 137.7	Control group (vehicle)	886.8 ± 193.5	825.6 ± 100.4
Acotiamide 3 mg/kg group	995.2 ± 235.1	728.9 ± 176.3	Itopride 30 mg/kg group	1494.7 ± 316.2*	1057.9 ± 198.3
Control group (vehicle)	787.3 ± 67.1	684.8 ± 154.5	Control group (vehicle)	698.8 ± 112.0	709.0 ± 142.1
Acotiamide 10 mg/kg group	1085.7 ± 238.5	934.8 ± 180.0*	Mosapride 10 mg/kg group	1618.5 ± 269.3**	1648.4 ± 110.8**
Control group (vehicle)	932.9 ± 222.6	715.5 ± 186.4			
Acotiamide 30 mg/kg group	2340.5 ± 473.4*	1233.8 ± 270.4*			

n = 6, mean ± standard error (SE)
*: $P < 0.05$, **: $P < 0.01$ (vs. each control group; paired t-test)

(b) Effect on postprandial gastrointestinal motility in dogs (4.2.1.1-21: Study 611 035)

In the study (a) above, SGTs were sutured onto the duodenum, ileum, and colon as well, and the effect of each drug on the postprandial motility of each site was investigated in a similar manner as with the gastric antrum. Acotiamide at ≥10 mg/kg significantly enhanced the postprandial duodenal motility (0-2 h) and colonic motility (0-2 h) compared with the vehicle. Itopride also significantly enhanced the postprandial ileal motility (0-2 h and 2-4 h), whereas mosapride had no significant effect on any of the regions.

The applicant explained that the relationship between the motility-enhancing effect of acotiamide on the duodenum and colon observed in this study and the clinical effect of the drug is unclear.

(c) Effect of repeated administration of acotiamide on postprandial antral motility in dogs (4.2.1.1-22: Study 620 203)

The vehicle (0.5 w/v% methylcellulose [MC] solution) or acotiamide (30 mg/kg) was administered intraduodenally once daily for 6 days to dogs with an SGT sutured onto the gastric antrum. At 2 to 4 hours after feeding on the next day of the last dose, a single dose of acotiamide (30 mg/kg) was administered intraduodenally to both the acotiamide group and vehicle group, and motility index ratio[2] was calculated from the motion waveform up to 60 minutes after acotiamide administration. The motility index ratio (mean ± standard error [SE]) was 220.4% ± 14.5% in the vehicle group and 223.7% ± 8.3% in the acotiamide group, showing no significant difference between the groups.

The applicant explained that repeated administration of acotiamide is unlikely to result in either enhancement or attenuation of its effect on the postprandial antral motility.

(d) Effect on clonidine-induced hypomotility of the gastric antrum in dogs (4.2.1.1-23: Study 620 205)

A single dose of the vehicle (10 w/v% gelatin solution) or clonidine hydrochloride (clonidine, 15 μg/kg) was administered subcutaneously to dogs with an SGT sutured onto the gastric antrum and, after 15 minutes, the vehicle (0.5 w/v% MC solution), acotiamide (3, 10, 30 mg/kg), itopride (30 mg/kg), or mosapride (10 mg/kg) was administered intraduodenally in a single

[2] The mean percentage of the motility index for 30 minutes after acotiamide administration relative to that obtained for 30 minutes before acotiamide administration

dose.[3] Motility index ratio[4] was calculated from the motion waveform up to 60 minutes after intraduodenal administration. The motility index ratios (mean ± SE) in the normal group (vehicle [10 w/v% gelatin solution] + vehicle [0.5 w/v% MC solution]), the control group (clonidine + vehicle [0.5 w/v% MC solution]), the acotiamide 3 mg/kg group, the acotiamide 10 mg/kg group, the acotiamide 30 mg/kg group, the itopride group, and the mosapride group were 96.0% ± 4.3%, 23.8% ± 4.2%, 24.9% ± 3.1%, 86.8% ± 7.6%, 138.7% ± 6.9%, 105.1% ± 10.5%, and 63.1% ± 5.1%, respectively, demonstrating that the reduction in the antral motility observed in the control group was significantly improved by acotiamide at ≥10 mg/kg, itopride, and mosapride.

(e) Effect on the antral motility in rats (4.2.1.1-24: Study 620[redacted]219)

A single dose of the vehicle (mixture of dimethyl sulfoxide and 5 w/v% glucose solution for injection [5:95] [mixed solution]), acotiamide (10, 30, 100 mg/kg), itopride (100 mg/kg), or mosapride (10 mg/kg) was administered subcutaneously to rats with an SGT sutured onto the gastric antrum, under fasted conditions. Table 4 shows the motility index ratio[5] during each 30 minutes calculated from the motion waveform up to 90 minutes after administration. Acotiamide at ≥30 mg/kg and itopride significantly enhanced the antral motility, whereas mosapride did not have any significant effect.

Table 4. Effect on the antral motility in rats

Treatment group	Motility index ratio (%)		
	0-30 min	30-60 min	60-90 min
Control group (vehicle)	94.0 ± 7.0	77.3 ± 6.8	98.9 ± 12.1
Acotiamide 10 mg/kg group	146.3 ± 18.6	126.5 ± 13.7	95.0 ± 9.8
Acotiamide 30 mg/kg group	248.0 ± 38.3***	242.5 ± 54.9***	175.6 ± 44.3*
Acotiamide 100 mg/kg group	242.4 ± 28.3***	255.4 ± 33.7***	172.0 ± 19.1*
Itopride 100 mg/kg group	190.0 ± 28.0*	186.1 ± 22.0*	166.1 ± 19.3
Mosapride 10 mg/kg group	69.9 ± 6.9	64.6 ± 6.8	75.1 ± 6.6

n = 10, mean ± SE
*: $P < 0.05$, ***: $P < 0.001$ (vs. control group; Dunnett test)

(f) Effect on clonidine-induced hypomotility of the gastric antrum in rats (4.2.1.1-25: Study 620[redacted]203)

A single dose of the vehicle (physiological saline) or clonidine (100 μg/kg) was administered subcutaneously to rats with an SGT sutured onto the gastric antrum and, after 30 minutes, the vehicle (mixed solution), acotiamide (10, 30, 100 mg/kg), itopride (100 mg/kg), or mosapride (10 mg/kg) was administered subcutaneously in a single dose. The motility index ratio[6] was calculated from the motion waveform up to 30 minutes after administration. The motility index ratios (mean ± SE) in the normal group (vehicle [physiological saline] + vehicle [mixed solution]), the control group (clonidine + vehicle [mixed solution]), the acotiamide 10 mg/kg group, the acotiamide 30 mg/kg group, the acotiamide 100 mg/kg, the itopride group, and the mosapride group were 87.7% ± 11.5%, 40.9% ± 6.0%, 74.4% ± 14.2%, 93.3% ± 21.2%, 127.5% ± 16.0%, 139.2% ± 35.7%, and 62.9% ± 9.0%, respectively. Thus, acotiamide at 100 mg/kg and itopride significantly improved the reduction in the antral motility observed in the control group,

[3] The vehicle (vehicle + vehicle) alone, clonidine (clonidine + vehicle) alone, and clonidine + acotiamide at each dose, itopride, or mosapride were administered to the same animal after a washout period of at least 5 days.

[4] The mean percentage of the motility index per 30 minutes up to 60 minutes after study drug administration relative to that obtained for 30 minutes before subcutaneous administration of clonidine or vehicle

[5] The percentage of the motility index during each period (30 minutes) after administration of each study drug relative to that obtained for 30 minutes before study drug administration

[6] The percentage of the motility index for 30 minutes after administration of study drug relative to that obtained for 30 minutes before administration of the vehicle or clonidine

whereas mosapride did not have any significant effect.

3.(i).A.(1).2) Effect on gastric emptying

(a) Effect on normal gastric emptying in rats (4.2.1.1-26: Study 620[redacted]203)

The vehicle (mixed solution), acotiamide (10, 30, 100 mg/kg), itopride (100 mg/kg), or mosapride (10 mg/kg) was administered subcutaneously in a single dose to rats under fasted conditions and, after 10 minutes, a liquid test diet containing 0.05 w/v% phenol red (PR) was loaded in a single oral dose. The gastric emptying rate[7] was calculated by the amount of PR remaining in the stomach at 15 minutes after feeding of the liquid test diet. The gastric emptying rate (mean ± SE) were 39.7% ± 3.0%, 45.1% ± 2.9%, 42.2% ± 3.1%, 37.3% ± 4.9%, 36.2% ± 3.5%, and 70.0% ± 3.1%, respectively. Thus, neither acotiamide nor itopride had any significant effect on the gastric emptying rate compared with the vehicle group, whereas mosapride significantly increased the gastric emptying rate.

(b) Effect on clonidine-induced delayed gastric emptying in rats (4.2.1.1-27: Study 620[redacted]204)

The vehicle (physiological saline) or clonidine (100 μg/kg) was administered subcutaneously in a single dose to rats under fasted condition and, after 5 minutes, the vehicle (mixed solution), acotiamide (10, 30, 100 mg/kg), itopride (100 mg/kg), or mosapride (10 mg/kg) was administered subcutaneously in a single dose. At 10 minutes after the administration, the liquid test diet containing 0.05% PR was loaded in a single oral dose, and the gastric emptying rate[7] was calculated by the amount of PR remaining in the stomach at 30 minutes after feeding of the liquid test diet. The gastric emptying rates (mean ± SE) in the normal group (vehicle [physiological saline] + vehicle [mixed solution]), the control group (clonidine + vehicle [mixed solution]), the acotiamide 10 mg/kg group, the acotiamide 30 mg/kg group, the acotiamide 100 mg/kg group, the itopride group, and the mosapride group were 68.7% ± 2.2%, 30.8% ± 2.5%, 32.1% ± 2.3% 39.1% ± 2.5%, 47.8% ± 3.0%, 48.9% ± 1.8%, and 28.4% ± 2.4%, respectively. Thus, acotiamide at 100 mg/kg and itopride significantly improved the reduced gastric emptying rate observed in the control group, whereas mosapride did not have any significant effect compared to the control group.

Regarding the findings that acotiamide improved clonidine-induced delay in gastric emptying but did not affect the normal gastric emptying, the applicant discussed that since under normal conditions, gastric motility is maintained at a sufficient level to allow emptying of the gastric content at a proper rate, further enhancement of the gastric motility may hardly lead to increase in gastric emptying.

3.(i).A.(1).3) Studies on the mechanism of action

(a) AChE inhibitory activity and the mode of inhibition (4.2.1.1-1–4.2.1.1-4: Study 620[redacted]210, 620[redacted]213, 620[redacted]214, 620[redacted]202)

Acotiamide inhibited recombinant human acetylcholinesterase (AChE) activity by a mixed type of inhibition comprising competitive inhibition (K_{i1}[8] [mean ± SE], 0.61 ± 0.03 μmol/L) and noncompetitive inhibition (K_{i2}[9], 2.7 ± 0.2 μmol/L). Itopride and neostigmine bromide (neostigmine) also inhibited AChE activity by a noncompetitive inhibition (K_i, 1.1 ± 0.0 μmol/L) and by a mixed type of inhibition (K_{i1}, 0.094 ± 0.004 μmol/L; K_{i2}, 0.26 ± 0.01 μmol/L), respectively.

In addition, acotiamide, itopride, and neostigmine inhibited AChE derived from the gastric

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[7] The percentage of PR remaining in the stomach in pylorus-ligated rats after oral loading of liquid test diet containing 0.05% PR, relative to the amount of PR present in the stomach immediately after loading

[8] Inhibition constant in competitive inhibition

[9] Inhibition constant in non-competitive inhibition

tissue of rats, guinea pigs, and dogs with IC_{50} of 1.2 to 3.6 μmol/L, 1.2 to 1.6 μmol/L, and 0.092 to 0.36 μmol/L, respectively. In contrast, mosapride inhibited AChE derived from dog gastric tissue only by 8.3% ± 1.3% (mean ± SE), even at 50 μmol/L, the maximum concentration tested.

(b) Selectivity and reversibility of AChE inhibition (4.2.1.1-5: Study 620[redacted]226)

The inhibitory activity of acotiamide, itopride, mosapride, neostigmine, and physostigmine hemisulfate (physostigmine) against recombinant human AChE and human Globulins Cohn fraction IV-4-derived butyrylcholinesterase (BuChE) was investigated. Table 5 shows IC_{50} values.

Table 5. IC_{50} against AChE and BuChE and inhibition ratio thereof

Test substance	IC_{50} [a] (μmol/L)		Inhibition ratio[b]
	Recombinant human AChE	Human Globulins Cohn fraction IV-4-derived BuChE	
Acotiamide	3.0	> 1000	> 330
Itopride	1.2	430	360
Mosapride	> 50	21	< 0.42
Neostigmine	0.21	2.4	11
Physostigmine	0.24	0.21	0.88

a) Calculated by a logistic curve fitted to the inhibition rate (mean of 4 samples) at each concentration by the non-linear least-squares method.
b) "IC_{50} against human Globulins Cohn fraction IV-4-derived BuChE" / "IC_{50} against recombinant human AChE"

In order to test the reversibility of AChE inhibition by acotiamide, the effect of dialysis on acotiamide-induced AChE inhibition was investigated. The inhibition rate (mean ± SE) of acotiamide (100 μmol/L) against recombinant human AChE in the non-dialyzed sample and in the dialyzed sample was 75.1% ± 1.4% and 1.0% ± 1.0%, respectively, which demonstrated that the AChE inhibitory effect of acotiamide was lost almost completely by dialysis.

The applicant explained that the above results suggested that acotiamide inhibits AChE more selectively than BuChE and that the inhibitory effect is reversible.

(c) Effect on acetylcholine- and carbachol-induced contraction of muscle strips isolated from guinea pig gastric antrum (4.2.1.1-6, 4.2.1.1-8: Study 611[redacted]034, 620[redacted]214)

The vehicle, acotiamide (0.3-3 μmol/L), itopride (3 μmol/L), or mosapride (10 μmol/L) was added to muscle strips isolated from guinea pig gastric antrum and, after 5 minutes, acetylcholine chloride (ACh[10]), 0.01 to 100 μmol/L, was added in a cumulative manner to evaluate the effect on ACh-induced contraction. Acotiamide significantly enhanced ACh (3, 30 μmol/L)-induced contraction at 1 μmol/L and ACh (0.1-100 μmol/L)-induced contraction at 3 μmol/L. Itopride also significantly enhanced ACh (1-100 μmol/L)-induced contraction, whereas mosapride did not have any significant effect.

The vehicle, acotiamide (3 μmol/L), itopride (3 μmol/L), or mosapride (10 μmol/L) was added to muscle strips isolated from guinea pig gastric antrum and, after 10 minutes, carbachol (CCh), 3 to 300 nmol/L, was added in a cumulative manner to evaluate the effect on CCh-induced contraction. Acotiamide, itopride, and mosapride did not affect CCh-induced contraction up to CCh concentration of 100 nmol/L, whereas they significantly attenuated the contraction induced by CCh 300 nmol/L.

[10] Hereinafter, "ACh" that appears together with added concentration (or dose) indicates "acetylcholine chloride", and "ACh" that appears alone indicates "acetylcholine."

(d) Effect on acetylcholine- and carbachol-induced contraction of muscle strips isolated from guinea pig gastric body (4.2.1.1-7, 4.2.1.1-9: Study 620[redacted]201, 620[redacted]202)

The vehicle, acotiamide (0.3-3 μmol/L), itopride (0.3-3 μmol/L), or neostigmine (3-30 nmol/L) was added to muscle strips isolated from guinea pig gastric body and, after 10 minutes, ACh (0.01-100 μmol/L) was added in a cumulative manner to evaluate the effect on ACh-induced contraction. Acotiamide significantly enhanced ACh (30, 100 μmol/L)-induced contraction at 0.3 μmol/L and ACh (1-100 μmol/L)-induced contraction at 1 and 3 μmol/L. Itopride significantly enhanced ACh (30, 100 μmol/L)-induced contraction at 1 μmol/L and ACh (10-100 μmol/L)-induced contraction at 3 μmol/L. Neostigmine significantly enhanced ACh (3-100 μmol/L)-induced contraction at 10 nmol/L and ACh (0.01-100 μmol/L)-induced contraction at 30 nmol/L.

The vehicle, acotiamide (3 μmol/L), itopride (3 μmol/L), or neostigmine (30 nmol/L) was added to muscle strips isolated from guinea pig gastric body and, after 10 minutes, CCh (3-1000 nmol/L) was added in a cumulative manner to evaluate the effect on CCh-induced contraction. None of the study drugs significantly affected CCh-induced contraction.

The applicant discussed as follows:
In the studies (c) and (d), acotiamide enhanced ACh-induced contraction of the gastric antrum and gastric body, but did not enhance the contraction by CCh which is less susceptible to degradation by cholinesterase. These results provided pharmacological evidence that the AChE inhibition is involved in the enhancement by acotiamide of ACh-induced contraction of the gastric antrum and gastric body.

The applicant also discussed as follows:
The reason for the finding that acotiamide attenuated the CCh (300 nmol/L)-induced contraction in the gastric antrum is unclear. However, since the extent of the attenuation was small (approx. 8.7%) and acotiamide did not attenuate ACh-induced contraction, the physiological significance of the effect is minor.

(e) Effect on acetylcholine-induced antral motility in dogs (4.2.1.1-10: Study 620[redacted]201)

An SGT was sutured onto the gastric antrum of a dog and, at 5 minutes after interdigestive migrating contraction of the gastric antrum was confirmed (resting phase), the vehicle (0.5 w/v% MC solution), acotiamide (3, 10 mg/kg), itopride (30 mg/kg), or neostigmine (1.5 mg/kg) was administered intraduodenally in a single dose.[11] From 15 minutes after the administration, ACh (0.05 mg/kg/min) was administered as a 5-minute continuous intravenous infusion. Table 6 shows the motility index calculated from the data obtained during the 5-minute continuous infusion. Acotiamide at both doses significantly increased the motility index, and both itopride and neostigmine significantly increased the motility index in a similar manner.

[11] Each dose of acotiamide, itopride, and neostigmine was administered to the same animal after a washout period of at least 5 days. The vehicle was administered 1 to 2 days before each administration.

Table 6. Effect on ACh-induced antral motility in dogs

Treatment group	Motility index (V·s)	Treatment group	Motility index (V·s)
Control group (vehicle)	20.8 ± 6.1	Control group (vehicle)	20.3 ± 5.8
Acotiamide 3 mg/kg group	65.3 ± 15.5*	Itopride 30 mg/kg group	66.8 ± 12.8**
Control group (vehicle)	20.4 ± 5.9	Control group (vehicle)	22.0 ± 6.0
Acotiamide 10 mg/kg group	106.8 ± 19.7**	Neostigmine 1.5 mg/kg group	115.8 ± 27.2**

n = 6, mean ± SE
*: $P < 0.05$, **: $P < 0.01$ (vs. each control group; paired t-test)

On the basis of the above results, the applicant considered that enhancement of ACh-induced antral motility observed in the *in vitro* study in (c) was confirmed in the *in vivo* study.

(f) Effect on electrically induced contraction of muscle strips isolated from guinea pig gastric body (4.2.1.1-11: Study 620[redacted]204)

The vehicle, acotiamide (0.1-1 μmol/L), itopride (0.3-3 μmol/L), or mosapride (1-10 μmol/L) was added to muscle strips isolated from guinea pig gastric body and, after 10 minutes, transmural electrical stimulation[12] (conditions; frequency of 1 Hz, pulse width of 1 msec, voltage of 10 V, 2-minute duration) was applied. Table 7 shows the effect on electrically induced contraction. Acotiamide at ≥0.3 μmol/L concentrations significantly enhanced the electrically induced contraction. Itopride at ≥1 μmol/L also significantly enhanced the contraction, whereas mosapride did not have any significant effect.

Table 7. Effect on electrically induced contraction of muscle strips isolated from guinea pig gastric body

Treatment group	Contraction ratio[a)]	Treatment group	Contraction ratio[a)]	Treatment group	Contraction ratio[a)]
Control group (vehicle)	0.96 ± 0.04	Control group (vehicle)	0.94 ± 0.04	Control group (vehicle)	0.94 ± 0.03
Acotiamide 0.1 μmol/L group	1.16 ± 0.05	Itopride 0.3 μmol/L group	1.23 ± 0.04	Mosapride 1 μmol/L group	1.08 ± 0.06
Acotiamide 0.3 μmol/L group	1.62 ± 0.12**	Itopride 1 μmol/L group	1.62 ± 0.14**	Mosapride 10 μmol/L group	1.11 ± 0.10
Acotiamide 1 μmol/L group	2.29 ± 0.19***	Itopride 3 μmol/L group	2.60 ± 0.30***		

n = 8, mean ± SE
a) Ratio of the maximum contractile force induced by electrical stimulation after study drug (or vehicle) addition to the maximum contraction force induced by electrical stimulation before study drug (or vehicle) addition
**: $P < 0.01$, ***: $P < 0.001$ (vs. each control group; nonparametric Dunnett test)

(g) Effect on gastric body contraction induced by electrical stimulation of the vagus nerve in rats (4.2.1.1-12, 4.2.1.1-13: Study 620[redacted]210, 620[redacted]203)

Electrical stimulation[13] (conditions; frequency of 5 Hz, pulse width of 1 msec, voltage of 20 V, 10-second duration) was applied continuously at 1-minute intervals to the gastric branches of the vagus nerve of anesthetized rats with an SGT sutured onto the gastric body, after which the vehicle (mixed solution) or acotiamide (3, 10, 30 mg/kg) was administered subcutaneously in a single dose. Table 8 shows the motility index ratio[14] during each 15-minute period calculated from the motion waveform from 15 minutes before administration up to 60 minutes after

[12] It is reported the contraction of muscle strips isolated from guinea pig gastric body by transmural electrical stimulation (frequencies of 2, 4, 8, and 16 Hz, pulse width of 0.5 msec, voltage of 100 V, 60-second duration) is a muscarinic receptor-mediated reaction induced by endogenous ACh (*Neurogastroenterol Motil.* 2004;16:143-153).

[13] It is reported that gastric body contraction induced by electrical stimulation of the vagus nerve in rats is a mescaline receptor-mediated reaction induced by endogenous ACh (*J Physiol.* 1995;484:481-492).

[14] The percentage of the motility index during each period (15 minutes) after administration of each study drug relative to that obtained for 15 minutes before study drug administration.

administration. Acotiamide at ≥10 mg/kg significantly increased the motility index ratio in all periods.

Table 8. Effect on gastric body contraction induced by electrical stimulation of the vagus nerve in rats

Treatment group	Motility index ratio (%)			
	0-15 min	15-30 min	30-45 min	45-60 min
Control group (vehicle)	89.1 ± 3.6	78.4 ± 5.7	71.2 ± 5.5	56.9 ± 3.6
Acotiamide 3 mg/kg group	96.7 ± 5.1	83.5 ± 8.5	75.0 ± 9.3	70.6 ± 9.6
Acotiamide 10 mg/kg group	115.7 ± 6.1**	137.2 ± 11.4**	133.8 ± 10.9**	113.9 ± 10.0**
Acotiamide 30 mg/kg group	125.0 ± 7.8***	188.2 ± 16.4***	194.5 ± 21.7***	172.4 ± 22.9***

n = 10, mean ± SE
**: $P < 0.01$, ***: $P < 0.001$ (vs. each control group; nonparametric Dunnett test)

In a separate experiment, electrical stimulation (conditions; frequency of 5 Hz, pulse width of 1 msec, voltage of 20 V, 10-second duration) was applied continuously at 1-minute intervals to the gastric branches of the vagus nerve of anesthetized rats with an SGT sutured onto the gastric body, after which the vehicle (mixed solution), acotiamide (30 mg/kg), itopride (10, 30 mg/kg), or mosapride (3, 10 mg/kg) was administered subcutaneously in a single dose. Mean motility index ratio[15] per 15 minutes was calculated from the motion waveform from pre-dose 15 minutes up to post-dose 60 minutes. The mean motility index ratios (mean ± SE) in the control (vehicle) group and the acotiamide group were 76.2% ± 3.9% and 160.6% ± 10%, respectively, showing a significant increase in the acotiamide group. The mean motility index ratios in the itopride 10 and 30 mg/kg groups were 96.1% ± 6.4% and 128.4% ± 7.6%, respectively, showing a significant increase in the 30 mg/kg group compared with the control (vehicle) group. In contrast, the mean motility index ratio in the mosapride 3 and 10 mg/kg groups was 79.7% ± 7.5% and 85.9% ± 6.4%, respectively, with mosapride showing no significant effect compared with the control (vehicle).

(h) Effect of atropine on the activity of acotiamide to enhance postprandial antral motility in dogs (4.2.1.1-14: Study 620[redacted]211)

Dogs with an SGT sutured onto the gastric antrum were fed, and then a single dose of the vehicle (0.5 w/v% MC solution) or acotiamide (30 mg/kg) was administered intraduodenally to the animals. At 30 minutes after the administration, the vehicle (physiological saline) or atropine sulfate (atropine, 0.05 mg/kg) was administered intravenously in a single dose, followed immediately by continuous intravenous administration of atropine (0.05 mg/kg/hr) or the vehicle (physiological saline). The motility index ratio[16] was calculated from the motion waveform up to 30 minutes after the start of the intravenous administration. The motility index ratios (mean ± SE) in the normal group (vehicle [0.5 w/v% MC solution] + vehicle [physiological saline]), the control group (acotiamide + vehicle [physiological saline]), and the atropine group (acotiamide + atropine) were 99.6% ± 8.3%, 209.7% ± 15.8%, and 15.9% ± 2.7%, respectively, demonstrating that atropine significantly suppressed the activity of acotiamide to enhance the antral motility observed in the control group.

The applicant explained as follows:
In the studies (f) to (h), the following findings were identified: (1) acotiamide enhanced the contraction of the gastric body induced by the electrical stimulation and by the stimulation of the vagus nerve that is considered to be caused by the muscarinic receptor-mediated action of

[15] The mean percentage of the motility index per 15 minutes up to 60 minutes after administration of each study drug relative to that obtained for 15 minutes before study drug administration
[16] The percentage of the motility index for 30 minutes after administration of atropine or the vehicle relative to that obtained for 30 minutes before administration of acotiamide or the vehicle

endogenous ACh; and (2) the activity of acotiamide to enhance the postprandial antral motility was suppressed by atropine, a muscarinic receptor antagonist. Therefore, the activity of acotiamide to enhance the contraction of the gastric body and to enhance the postprandial antral motility is likely to be mediated by muscarinic receptor.

(i) Affinity to muscarinic, dopamine, and serotonin receptors (4.2.1.1-15–4.2.1.1-19: Study 611[redacted]001, 611[redacted]002, 611[redacted]003, 611[redacted]036, 620[redacted]223)

The activity of acotiamide, itopride, and mosapride to inhibit the binding of labeled ligands to recombinant human muscarinic M_1, M_2, M_3 receptors, dopamine D_{2S} receptor, guinea pig striatum-derived serotonin 5-HT_4 receptor, and recombinant human serotonin 5-HT_{4c}, 5-HT_{4d}, and 5-HT_{4e} receptors was investigated. Table 9 shows K_i values.

Table 9. K_i values against muscarinic, dopamine, and serotonin receptors

Receptor	K_i (μmol/L)		
	Acotiamide	Itopride	Mosapride
Recombinant human muscarinic M_1	27 a)	67 a)	14 a)
Recombinant human muscarinic M_2	31 a)	33 a)	96% a) b)
Recombinant human muscarinic M_3	270 a)	69% a) b)	75% a) b)
Recombinant human dopamine D_{2S}	> 100	3.7	14
Guinea pig striatum-derived serotonin 5-HT_4	> 100	57% b)	0.067
Recombinant human serotonin 5-HT_{4c}	> 100	> 100	0.18
Recombinant human serotonin 5-HT_{4d}	> 100	> 100	0.13
Recombinant human serotonin 5-HT_{4e}	> 100	> 100	0.14

n = 3, mean
a) n = 1
b) Inhibition rate at 100 μmol/L

Based on the finding that acotiamide had only low affinity for receptors that are known to be involved in the regulation of the gastrointestinal motility, the applicant discussed that the enhancement by acotiamide of ACh-induced contraction and motility of the gastric antrum and gastric body is mainly due to its AChE inhibitory activity.

(j) Effect on other receptors, etc. (4.2.1.1-20, 4.2.1.1-28: Study 620[redacted]202, 620[redacted]202)

The inhibitory effect of acotiamide at 1, 10, and 100 μmol/L against 41 different types of receptors was investigated. Results showed that acotiamide inhibited the binding of the following receptors by ≥50%: guinea pig-derived adrenaline β_2 receptor (inhibition rate 51%) by acotiamide 10 μmol/L; and rat-derived adenosine A_{2A} (83%), dopamine D_2 (86%), dopamine D_3 (59%), $GABA_A$ (66%), muscarinic M_1 (97%), M_2 (93%) receptors, guinea pig-derived adrenaline β_2 receptor (92%), and recombinant human muscarinic M_4 receptor (79%) by acotiamide 100 μmol/L.

In a separate experiment, the inhibitory effect of acotiamide at 10 μmol/L against 47 different types of receptors, 5 types of ion channels, 3 types of transporters, and 3 types of enzymes was investigated. Acotiamide inhibited the following receptors, etc., by ≥20%: adenosine A_1 (40.34%), adrenaline β (37.34%), and sodium channel site 2 (53.09%) (rat-derived); and 5-HT_{2B} receptor (42.06%) and AChE (27.83%) (recombinant human).

3.(i).A.(1).4) Pharmacological action of metabolites

(a) Effect of metabolites on receptors (4.2.1.1-28: Study 620[redacted]202)

The inhibitory activities of the glucuronide conjugate of acotiamide (M-1, 10 μmol/L) and the glucuronide conjugate of deisopropyl acotiamide (M-2, 10 μmol/L), the major metabolites of acotiamide in human plasma [see "3.(ii).A.(3).5) Studies on metabolites in plasma, urine, and feces of humans"], against 47 types of receptors, 5 types of ion channels, 3 types of transporters,

and 3 types of enzymes were investigated. Neither M-1 nor M-2 inhibited any of the receptors, etc., by ≥25%.

On the basis of the above results, the applicant discussed that the glucuronide conjugate of acotiamide (M-1) and the glucuronide conjugate of deisopropyl acotiamide (M-2) are unlikely to have pharmacological activity.

3.(i).A.(2) Secondary pharmacodynamics

3.(i).A.(2).1) Effect on basal gastric juice secretion in rats (4.2.1.2-1: Study 620[redacted]211)

Physiological saline was administered intravenously continuously for 5 hours to rats with an indwelling gastrostomy tube under fasted conditions. At 1 hour after the start of the administration of physiological saline, the vehicle (mixed solution) or acotiamide (1, 10, 100 mg/kg) was administered subcutaneously in a single dose, and gastric juice was collected every hour and acid output was calculated. Table 10 shows the results. Acotiamide at 100 mg/kg caused a significant increase in acid output at 1 and 2 hours after administration, but had no significant effect at 3 and 4 hours after administration.

Table 10. Effect on basal gastric juice secretion

Treatment group	Acid output (μEq/h)				
	Before s.c. administration	After s.c. administration			
		1h	2h	3h	4h
Control group (vehicle)	33.9 ± 8.6	17.3 ± 7.9	27.1 ± 7.7	44.0 ± 10.1	62.2 ± 23.7
Acotiamide 1 mg/kg group	32.7 ± 6.6	35.2 ± 9.8	32.1 ± 9.5	34.9 ± 10.0	49.2 ± 12.5
Acotiamide 10 mg/kg group	29.6 ± 6.5	46.5 ± 8.6	31.6 ± 8.4	50.5 ± 14.7	67.1 ± 11.9
Acotiamide 100 mg/kg group	34.0 ± 6.1	78.3 ± 10.6***	61.2 ± 10.5*	49.6 ± 11.7	52.8 ± 15.8

n = 8, mean ± SE
*: $P < 0.05$, ***: $P < 0.001$ (vs. control group; Dunnett test)

The applicant considered as follows:
The increase in acid secretion was possibly caused by the increased effect of endogenous ACh due to AChE inhibition by acotiamide. Also, since no histopathological changes were observed either in the stomach or in the esophagus in the repeated-dose toxicity studies [see "3.(iii).A.(2) Repeated-dose toxicity"], the acotiamide-induced increase in acid secretion is unlikely to cause any serious safety concerns in humans.

3.(i).A.(3) Safety pharmacology

3.(i).A.(3).1) Effect on central nervous system (4.2.1.3-1: Study 611[redacted]509)

The vehicle (0.5 w/v% MC solution) or acotiamide (10, 100, 1000 mg/kg) was administered in a single oral dose to rats, and the effect on general symptoms and behavior was evaluated using the functional observational battery. Miosis was observed within 4 hours after administration in the acotiamide ≥100 mg/kg groups, whereas no effect was observed on other observation parameters.

Following the oral administration of acotiamide (10 mg/kg) to rats, the estimated plasma concentration of unbound acotiamide was 63.4 to 70.1 ng/mL,[17] which was 3.7- to 4.6-fold the estimated plasma concentration of unbound acotiamide following the administration of the clinical dose to humans.[18]

[17] Calculated from plasma acotiamide concentration (282.1 ng/mL) following a single oral dose of acotiamide (10 mg/kg) to rats and from the protein binding rate (75.16%-77.52%) in rat plasma in the *in vitro* study.

[18] Calculated from the maximum plasma acotiamide concentration (109.0 ng/mL) during the multiple administration period in the phase I multiple dose study ([redacted]) orally administering acotiamide (100 mg) three times daily to healthy adult male subjects (duration of multiple administration, 7 days) and from the protein binding rate (84.21%-85.95%) in human plasma in the *in vitro* study.

On the basis of the finding that acotiamide is little distributed in the brain, the applicant considered that the observed miosis was a peripheral effect of acotiamide due to the AChE inhibition.

3.(i).A.(3).2) Effect on respiratory system (4.2.1.3-2: Study 611[redacted]508)

The vehicle (0.5 w/v% MC solution) or acotiamide (10, 100, 1000 mg/kg) was administered in a single oral dose to unanesthetized rats, and the effect on respiratory function (respiratory rate, tidal volume, minute ventilation, and enhanced pause [index of bronchoconstriction]) was investigated. Acotiamide had no effect on the respiratory function.

3.(i).A.(3).3) Effect on cardiovascular system

(a) *In vitro* studies

i) Effect on hERG channel (4.2.1.3-3: Study 621[redacted]075)

The vehicle or acotiamide (0.1-100 μmol/L) was added to HEK293 cells engineered to stably express hERG (human ether-a-go-go-related gene) channel, and the effect of acotiamide on hERG current was investigated using the whole cell patch-clamp method. Acotiamide 100 μmol/L significantly suppressed the hERG current compared with the vehicle, with the relative tail current (ratio to baseline, mean ± SE) in the vehicle group and the acotiamide 100 μmol/L group being 0.95 ± 0.02 and 0.57 ± 0.04, respectively.

In contrast, the positive control cisapride (3-100 nmol/L) suppressed hERG current in a concentration-dependent manner, with IC_{50} of 29.3 nmol/L. Acotiamide 100 μmol/L is ≥2500-fold the estimated concentration of unbound acotiamide in plasma[18] following the administration of the clinical dose to humans.

ii) Effect on rapidly activated delayed rectifier potassium current in rabbit ventricular muscle cells (non-GLP study) (4.2.1.3-4: Study 611[redacted]025)

Acotiamide (3-100 μmol/L) was added to isolated rabbit ventricular muscle cells, and the effect of acotiamide on rapidly activated delayed rectifier potassium current (I_{Kr}) was investigated using the whole-cell patch-clamp method. Acotiamide suppressed I_{Kr} in a concentration-dependent manner, with IC_{50} of 54 μmol/L. On the other hand, the positive control cisapride (3-300 nmol/L) suppressed I_{Kr} in a concentration-dependent manner, with IC_{50} of 42 nmol/L.

The acotiamide 54 μmol/L was ≥1400-fold the estimated concentration of unbound acotiamide in plasma following the administration of the clinical dose to humans.[18]

iii) Effect on action potential of guinea pig papillary muscle strips (4.2.1.3-5: Study 620[redacted]217)

Acotiamide (0.1-100 μmol/L) was added to guinea pig papillary muscle strips, and the effect on cardiac action potential parameters (resting membrane potential, action potential amplitude, maximum upstroke velocity, and the action potential duration at 50% and 90% repolarization [APD_{50}, APD_{90}]) was investigated. Acotiamide (100 μmol/L) significantly increased APD_{50} (111.1% relative to baseline) compared with the vehicle, and tended to increase APD_{90} as well (110.2% relative to baseline). On the other hand, the positive control cisapride (1 μmol/L) significantly increased APD_{50} (115.0% relative to baseline) and APD_{90} (117.7% relative to baseline) compared with the vehicle.

(b) *In vivo* studies

i) Effect on cardiovascular system in unanesthetized dogs (4.2.1.3-6: Study 621[redacted]082)

The vehicle (empty capsule) or acotiamide (10, 100, 1000 mg/kg) was administered in a single

oral dose to unanesthetized dogs,[19] and the effect of acotiamide on general symptoms, blood pressure, heart rate, and electrocardiogram (ECG) (PR, QRS, QT, RR, and QTc intervals) was investigated by telemetry. Changes in general symptoms observed were vomiting at ≥100 mg/kg dose of acotiamide and both vomiting and salivation at 1000 mg/kg. The rate of change in QT interval from baseline significantly increased at 10 mg/kg of acotiamide compared with the vehicle group, whereas acotiamide had no significant effect on other ECG parameters including QTc interval (Fridericia's corrections). Acotiamide (100 mg/kg) significantly decreased diastolic blood pressure and rate of change in heart rate from baseline. Acotiamide (1000 mg/kg) significantly decreased the rate of change in RR interval from baseline. The estimated concentration of unbound acotiamide in plasma following oral administration of acotiamide (10 mg/kg) to dogs was 327.2 to 391.2 μmol/L,[20] which was 19.0- to 25.5-fold the estimated concentration of unbound acotiamide in plasma following the administration of the clinical dose to humans.[18]

The applicant considered as follows:
Salivation, vomiting, decreased diastolic blood pressure, and decreased heart rate are possibly caused by AChE inhibitory activity of acotiamide. Also, based on the finding that the timing of the decrease in RR interval was close to that of vomiting (3 of 4 animals), the decrease in RR interval was possibly caused by tachycardia induced by vomiting.

ii) Effect on epicardial monophasic action potential duration in anesthetized guinea pigs (4.2.1.3-7: Study 611[redacted]551)

Acotiamide (0.1-10 mg/kg) was administered intravenously to anesthetized guinea pigs in a cumulative manner and, after administration of each dose, epicardial monophasic action potential duration at 70% and 90% of repolarization ($MAPD_{70}$, $MAPD_{90}$) in sinus rhythm, blood pressure, heart rate, and ECG (RR, QT, QTcB, and QTcF intervals) were measured. The rate of change in $MAPD_{70}$, $MAPD_{90}$, and QT interval from baseline were also determined after administration of each dose under cardiac pacing. The control group received intravenous administration of the vehicle (5 w/v% glucose solution) repeatedly. Measurement under sinus rhythm showed significant increase in $MAPD_{70}$ and $MAPD_{90}$, prolongation of QT, QTcB, and QTcF intervals, significant increase in blood pressure, and tendency of decrease in heart rate after acotiamide (10 mg/kg) administration compared with the control group. However, measurement under cardiac pacing did not show any significant effect on the rates of change in $MAPD_{70}$, $MAPD_{90}$, or QT intervals from baseline. In contrast, cisapride (0.01-1 mg/kg) prolonged $MAPD_{70}$ and $MAPD_{90}$ and increased the rate of change in QT interval from baseline in a dose-dependent manner under cardiac pacing. When acotiamide (10 mg/kg) was administered intravenously to a different group of guinea pigs, the mean plasma acotiamide concentration (in 6 animals) at 2 minutes after administration was 36,181 ng/mL, which was approximately 332-fold the plasma acotiamide concentration following the administration of the clinical dose to humans.[21]

iii) Torsade de Pointes-inducing effect in arrhythmogenic rabbit model (4.2.1.3-8: Study 611[redacted]550)

Acotiamide (30 mg/kg), clofilium tosylate (positive control, 1.9 μmol/kg), or cisapride (9 μmol/kg) was administered intravenously in a single dose over 30 minutes to anesthetized

[19] The vehicle or acotiamide (10, 100, 1000 mg/kg) was administered to the same animal after a washout period of at least 6 days.

[20] Calculated from plasma acotiamide concentration (839.4 ng/mL) following a single oral dose at 10 mg/kg to dogs and the protein binding rate (53.39%-61.02%) in dog plasma in *in vitro* study

[21] The maximum plasma acotiamide concentration (109.0 ng/mL) during multiple administration in the phase I multiple oral dose study ([redacted]) orally administering acotiamide (100 mg) three times daily to healthy adult male subjects (duration of multiple administration, 7 days).

arrhythmogenic rabbit model.[22] As a result, acotiamide had no effect on RR, QT, or QTc interval.[23] Neither did it induce ventricular extrasystoles (VPC), ventricular tachycardia (VT), or Torsade de Pointes (TdP). In contrast, clofilium tosylate prolonged QT and QTc intervals and induced VPC (6 of 6 animals), VT (2 of 6 animals), and TdP (2 of 6 animals). Cisapride shortened RR interval, prolonged QT and QTc intervals, and induced VPC (3 of 6 animals), but did not induce VT or TdP. Acotiamide (30 mg/kg) was administered to a different group of rabbits (2 animals) intravenously. The maximum plasma acotiamide concentrations in individual animals were 50,609 and 67,039 ng/mL, respectively, which were 464- and 615-fold the plasma acotiamide concentration observed following the administration of the clinical dose to humans[21], respectively.

3.(i).A.(3).4 Effect on renal function (4.2.1.3-9: Study 620[redacted]218)

After unanesthetized rats were forced to void, the vehicle (0.5 w/v% MC solution) or acotiamide (10, 100, 1000 mg/kg) was administered in a single oral dose, immediately after which physiological saline was loaded orally and urine was collected up to 6 hours after the loading. Acotiamide up to 1000 mg/kg did not affect urine output, urinary potassium output, or urinary osmotic pressure, whereas the 100 mg/kg group showed a significant increase in urinary sodium output and a tendency of increase in sodium-to-potassium concentration ratio, and the 1000 mg/kg group showed a tendency of increase in urinary sodium output and a significant increase in urinary chloride output.

The applicant discussed that increased urinary sodium and chloride output may possibly be caused by the inhibition of AChE, although the detailed mechanisms are unknown.

3.(i).A.(3).5 Effect on tracheal smooth muscles (4.2.1.3-10: Study 620[redacted]219)

The vehicle or acotiamide (0.1-100 μmol/L) was added to isolated guinea pig trachea in a cumulative manner. As a result, acotiamide alone had no effect and did not affect the contractile response induced by histamine dihydrochloride (histamine, 30 μmol/L).

3.(i).A.(3).6 Effect on skeletal muscles (4.2.1.3-11: Study 620[redacted]220)

The vehicle or acotiamide (10, 100, 1000 mg/kg) was administered intraduodenally in a single dose to anesthetized rats. Acotiamide had no effect on the contractile response of the gastrocnemius muscle induced by sciatic nerve stimulation.

3.(i).A.(4) Pharmacodynamic drug-drug interactions

3.(i).A.(4).1 Effect of acotiamide on acid secretion inhibitor-induced suppression of acid secretion (4.2.1.4-1: Study 620[redacted]207)

Histamine (8 mg/kg/hr) was intravenously administered continuously for 5 hours to rats with an indwelling gastrostomy tube under fasted condition. At 1 hour after the start of histamine administration, the vehicle (mixed solution) or acotiamide (1, 10, 100 mg/kg) was administered subcutaneously in a single dose and, at the same time, famotidine (10 mg/kg), lansoprazole (3 mg/kg), or the vehicle (0.5 w/v% MC solution) was administered intraduodenally in a single dose. Gastric juice was collected every hour and the total acid output from 1 to 4 hours after the combined administration was calculated. Table 11 shows the results. Acotiamide at any dose did not significantly affect the suppressive effect of each gastric acid secretion inhibitor on histamine-induced gastric acid secretion.

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[22] The arrhythmogenic model was prepared by continuous intravenous administration of methoxamine (α_1-adrenergic agonist) to anesthetized rabbits. It is reported that Torsade de Pointes is induced by some drugs that cause QT prolongation (*J Cardiovasc Pharmacol*. 1990;16:276-285).

[23] The value corrected for the effect of the heart rate by fitting the correction formula (QTc = QT – "slope" × [RR-500]) to the slope of the relationship between QT-RR intervals obtained from baseline values after methoxamine administration

Table 11. Effect on the suppression of acid secretion by acid secretion inhibitors

Treatment group	No. of animals	Total acid output (μEq/3h)	Suppression rate[a)] (%)
Normal group (vehicle + vehicle)	9	1060.2 ± 103.8	–
Control group (vehicle + famotidine)	9	86.0 ± 21.9***	91.9
Acotiamide 1 mg/kg + famotidine group	8	101.9 ± 24.6	90.4
Acotiamide 10 mg/kg + famotidine group	8	84.6 ± 24.3	92.0
Acotiamide 100 mg/kg + famotidine group	8	102.8 ± 15.0	90.3
Normal group (vehicle + vehicle)	8	806.7 ± 82.1	–
Control group (vehicle + lansoprazole)	7	96.4 ± 32.1***	88.1
Acotiamide 1 mg/kg + lansoprazole group	8	60.4 ± 13.5	92.5
Acotiamide 10 mg/kg + lansoprazole group	7	74.2 ± 31.7	90.8
Acotiamide 100 mg/kg + lansoprazole group	7	123.9 ± 32.3	84.6

Mean ± SE

***: $P < 0.001$ (vs. normal group; Welch test)

a) (["mean total acid output in normal group" - "mean total acid output in each group"] / "mean total acid output in the normal group" × 100

3.(i).A.(4).2) Effect of acid secretion inhibitors on the antral motility-enhancing effect of acotiamide (4.2.1.4-2: Study 620[redacted]204)

The vehicle (physiological saline), famotidine (0.3 mg/kg), or lansoprazole (0.3 mg/kg) was administered subcutaneously in a single dose to rats with an SGT sutured onto the gastric antrum, immediately after which the vehicle (mixed solution) or acotiamide (30 mg/kg) was administered subcutaneously in a single dose, and the motility index ratio[24] per 30 minutes was calculated from the motion waveform from pre-dose 30 minutes up to post-dose 60 minutes. As a result, the motility index ratios (mean ± SE) in the vehicle group (vehicle [physiological saline] + vehicle [mixed solution]), the control group (acotiamide + vehicle [physiological saline]), the concomitant use with famotidine group (acotiamide + famotidine), and the concomitant use with lansoprazole group (acotiamide + lansoprazole) were 97.6% ± 2.1%, 198.9% ± 15.2%, 195.9% ± 20.7%, and 214.0% ± 17.7%, respectively. Thus, neither famotidine nor lansoprazole affected the activity of acotiamide to enhance the antral motility.

On the basis of the above results, the applicant considered that the concomitant use of acotiamide with acid secretion inhibitors is unlikely to cause pharmacodynamic interactions.

3.(i).B Outline of the review by PMDA

3.(i).B.(1) Efficacy

The applicant explained the efficacy of acotiamide in treatment of functional dyspepsia (FD), as follows:

It is assumed that FD is caused by abnormal gastrointestinal motility (e.g., abnormal gastric emptying, gastric dysrhythmia, antral hypomotility, motility disorder of the small intestine), gastrointestinal hypersensitivity (e.g., gastroduodenal hypersensitivity and acid hypersensitivity), stress, brain-gut interaction (e.g., vagus nerve disorder, psychological distress, central nervous system disorder), impaired postprandial relaxation of gastric fundus, but their relationship with symptoms is unknown in most cases (e.g., *Dig Dis.* 2008;26: 194-202). It is known that patients with FD have functional gastrointestinal disorder, such as antral hypomotility and delayed gastric emptying. Therefore, it is expected that administering a drug that enhances the motility of the gastrointestinal tract and thereby improving the functional gastrointestinal disorder will serve as a treatment for FD.

The results of the studies on the primary pharmacodynamics of acotiamide, which are included

[24] The mean percentage of the motility index per 30 minutes up to 60 minutes after study drug administration relative to that obtained for 30 minutes before study drug administration.

in the data package submitted, have demonstrated that acotiamide enhances the postprandial gastric motility and that, in the clonidine-induced gastric hypomotility model, acotiamide improves the antral hypomotility and the delayed gastric emptying. These results raise the expectation that acotiamide is effective in patients with FD.

PMDA asked the applicant to explain the reason for using this model in evaluating the efficacy of acotiamide, by referring to the relationship between the pathology of FD and clonidine-induced gastric hypomotility model.

The applicant responded as follows:
It is reported that the pathology of FD is associated with the functional gastrointestinal disorder, such as antral hypomotility and delayed gastric emptying (*Am J Gastroenterol.* 2004;99: 739-749, *Neurogastroenterol Motil.* 2008;20: 618-624). Also, it is reported that the function of the vagus nerve may be decreased in patients with FD (*Psychosom Med.* 1994;56:181-186, *Psychosom Med.* 1993;55:12-22), which suggests a decrease of ACh released from the nerve terminal in patients with FD. Administration of clonidine to rats induced functional gastrointestinal disorder, such as antral hypomotility and delayed gastric emptying, which are symptoms similar to those observed in patients with FD. It is inferred that clonidine reduces gastric motility by stimulating adrenaline α_2 receptor present on the vagus nerve terminal, thereby decreasing the release of ACh from the vagus nerve terminal (*J Pharmacol Exp Ther.* 1993;266:1190-1195, *J Pharmacol Exp Ther.* 1998;287:712-719, *Eur J Pharmacol.* 2005;528: 150-157).

The above findings suggest that the clonidine-induced gastric hypomotility model is an animal model that represents a condition of decreased ACh release from the vagus nerve terminal, i.e., the condition that simulates the decreased vagus nerve function in patients with FD, thereby reflecting the gastric hypomotility in these patients. On the basis of the above, the model was used in efficacy evaluation of acotiamide.

PMDA considers as follows:
The pathology of FD has not been fully elucidated at the current moment, and patients with FD do not necessarily have antral hypomotility or delayed gastric emptying induced by decreased vagus nerve function. However, given that acotiamide improved antral hypomotility and delayed gastric emptying, which are considered to be part of the causes of FD, and that acotiamide enhanced postprandial antral motility, the efficacy of acotiamide in treatment of FD is suggested by the results of the primary pharmacodynamic studies submitted. However, since FD is considered to be a disease caused not only by the decreased antral motility and the delayed gastric emptying but also by other multiple causes in combination, it is recommended to investigate the effect on failure of gastric adaptive relaxation and gastrointestinal hypersensitivity in the future.

3.(i).B.(2) Mechanism of action of acotiamide

The applicant explained the relationship between the mechanism of action of acotiamide and the clinical efficacy, as follows:
The results on the mechanism of action from the primary pharmacodynamic studies submitted suggest that the AChE inhibition by acotiamide suppresses the degradation of ACh released from cholinergic nerve terminals, which results in enhancement of ACh-induced contraction and motility of the gastric antrum and gastric body, thereby increasing postprandial antral motility and improving antral hypomotility and delayed gastric emptying.

The plasma concentration of unbound acotiamide following the administration of the clinical

dose to humans is estimated to be ranging from 0.014 to 0.038 μmol/L.[25] Since there is a discrepancy between this concentration and K_{i1} of acotiamide against recombinant human AChE (0.61 μmol/L), the relationship between AChE inhibitory effect and the clinical results cannot be thoroughly interpreted from the plasma concentration of unbound acotiamide alone. However, studies on the distribution of acotiamide in the gastric tissue in rats suggest that acotiamide is incorporated into the gastric tissue from the circulating blood via an active uptake mechanism, which indicates the possibility that concentration of unbound acotiamide in the gastric tissue has reached the level sufficient for inhibiting AChE. Thus, it is inferred that the clinical efficacy of acotiamide is based on its AChE inhibitory activity.

PMDA considers as follows:
Considering that there is a discrepancy between the estimated plasma concentration of unbound acotiamide following the administration of the clinical dose and the inhibitory concentration against recombinant AChE in the *in vitro* study, and that extrapolability to humans of the acotiamide distribution in the gastric tissue observed in rats is unclear [see "3.(ii).B.(1) Transfer and distribution of acotiamide in gastric tissue"], there is currently no clear interpretation of the relationship between the AChE inhibitory activity of acotiamide and the clinical effect. However, since there are no major inconsistencies in the explanation of the applicant on the mechanism of action of acotiamide based on the results of the nonclinical studies, PMDA understands, to some extent, the applicant's view that acotiamide exhibits clinical efficacy via its AChE inhibitory activity.

3.(i).B.(3) Effects possibly elicited by AChE inhibition
The applicant considered as follows:
Regarding the effects of acotiamide possibly caused by increased ACh level due to the AChE inhibition (miosis, vomiting, salivation, decreased diastolic blood pressure, decreased heart rate, increased urinary electrolyte excretion) observed in the safety pharmacology studies, they are unlikely to pose clinical problems since (i) there is a discrepancy between the plasma acotiamide concentration following the administration to animals of the maximum dose at which the effects were not observed and the plasma concentration following the administration of the clinical dose to humans, and (ii) in the clinical studies, acotiamide administration did not increase the incidence of adverse events related to these effects.

Regarding enhancement of gastric acid secretion observed in the secondary pharmacodynamic studies, results of the clinical studies did not show any increase in the incidence of adverse events that are induced by this effect (e.g., reflux oesophagitis, gastric ulcer, upper abdominal pain, abdominal discomfort), neither did the toxicological studies show any histopathological changes in the esophagus, stomach, or duodenum. Therefore, the enhancement of gastric acid secretion is unlikely to have any clinical problems.

PMDA considers as follows:
There is no major problem with the applicant's explanation, and the applicant's view that at the current moment, there are no major safety concerns related to AChE inhibitory effect of acotiamide is understandable. However, when the effective dose of acotiamide in the clonidine-induced gastric hypomotility model and the maximum dose at which the actions were not observed in the safety pharmacology studies are compared, the comparison is difficult in rats because of the difference in the route of administration, whereas in dogs, the effective dose was the same as the maximum dose that did not cause vomiting or cardiovascular effect (10 mg/kg). In addition, considering that AChE is expressed throughout the body and that it is

[25] Calculated from the plasma concentration of unchanged acotiamide and the binding rate to human plasma proteins following the multiple oral administration of cotiamide 100 mg three times daily to healthy adult male subjects in the phase I multiple dose study ([redacted]) (duration of multiple administration, 7 days).

unclear whether or not the action of acotiamide is specific to the stomach, it cannot be excluded that events caused by cholinergic action mediated by AChE inhibition may occur when acotiamide is used in clinical settings. Therefore, it is necessary to collect information on events induced by cholinergic action and safety in patients to whom acotiamide is administered concomitantly with cholinergic drugs or anticholinergic drugs, which are included in drugs for which precautions for concomitant use are required [see "4.(iii).B.(9) Post-marketing surveillance, etc." for the details of the post-marketing surveillance, etc.].

3.(ii) Summary of pharmacokinetic studies

3.(ii).A Summary of the submitted data

The pharmacokinetics of acotiamide was determined following oral or intravenous administration of acotiamide or ^{14}C-labeled acotiamide to mice, rats, and dogs. In *in vitro* studies, distribution in blood cells, plasma protein binding rate, transcellular transport, metabolism, and drug interactions of acotiamide in various animal species and humans were also determined.

The concentration of unchanged acotiamide in samples was measured as acotiamide. Plasma acotiamide concentrations in rats and dogs were measured by high performance liquid chromatography with ultraviolet detector (HPLC-UV). The lower limit of quantitation was 5 ng/mL for rats and 2.5 ng/mL for dogs. In a part of the studies, acotiamide concentrations in plasma, gastric tissue, and skeletal muscle were measured by high performance liquid chromatography-tandem mass spectrometry (LC/MS/MS). The lower limit of quantitation was 1 ng/mL. In studies using ^{14}C-labeled acotiamide, radioactivity was determined by liquid scintillation method or by high performance liquid chromatography with radioisotope detector (HPLC-RI).

Male animals were used in *in vivo* studies, unless otherwise specified. In all studies, acotiamide dose is expressed in terms of the salt.

3.(ii).A.(1) Absorption

3.(ii).A.(1).1) Single-dose administration studies (4.2.2.1-1, 4.2.2.2-1–6: Study 604[redacted]020, 611[redacted]014, 516[redacted]229, 604[redacted]028, 604[redacted]013, 604[redacted]027, 622[redacted]009)

Table 12 shows the pharmacokinetic parameters of unchanged acotiamide in the plasma observed following a single oral or intravenous dose of acotiamide (3-30 mg/kg) to rats and dogs under fasted conditions, and following a single oral dose of acotiamide (10 mg/kg) to fasted or fed dogs. Table 13 shows the pharmacokinetic parameters of the radioactivity in the blood and in the plasma following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to fasted mice and dogs and to fasted or fed male and female rats.

Table 12. Pharmacokinetic parameters of unchanged acotiamide in the plasma following a single intravenous or oral dose of acotiamide

	Feeding condition	Route of administration	Dose (mg/kg)	No. of animals	C_{max} (ng/mL)	$AUC_{0-\infty}$ (ng·h/mL)	t_{max} (h)	$t_{1/2}$ (h)	CL/F (L/h/kg)	Vd/F (L/kg)	BA (%)
Rats[a)]	Fasted	i.v.	3	5/time point	–	586.3	–	0.8	4.3	4.9	–
		p.o.	3	5/time point	75.6	81.5	0.08	2.1	30.6	94.9	13.9
			10	5/time point	282.1	271.5	0.08	3.7	30.7	165.6	13.9
			30	5/time point	840.9	1115.7	0.08	3.0	22.4	97.2	19.0
Dogs	Fasted	i.v.	3	5	–	1179.6 ± 53.4	–	1.3 ± 0.2	2.1 ± 0.1	4.2 ± 0.7	–
		p.o.	3	5	138.2 ± 15.6	335.8 ± 68.2	0.4 ± 0.1	2.5 ± 0.6	8.6 ± 1.5	28.9 ± 6.1	27.8 ± 4.6
			10	5	839.4 ± 139.5	1470.5 ± 147.8	0.5 ± 0.1	3.0 ± 0.4	6.0 ± 0.8	26.5 ± 5.5	37.5 ± 3.6
			30	5	2643.3 ± 317.0	5997.3 ± 889.6	0.4 ± 0.1	2.9 ± 0.8	4.5 ± 0.5	19.0 ± 6.3	50.4 ± 5.8
Dogs	Fasted	p.o.	10	5	1070.2 ± 148.1	1968.9 ± 225.0[b)]	0.45 ± 0.05	–	–	–	–
	Fed		10	5	663.4 ± 205.8	1094.8 ± 252.2[b)]	0.08 ± 0.12	–	–	–	–

mean ± SE

a) Each parameter was calculated from the mean plasma concentration in 5 animals at each measuring time point.

b) AUC_{last}

Table 13. Pharmacokinetic parameters following a single oral dose of ^{14}C-labeled acotiamide

	Feeding condition	Test sample	Dose (mg/kg)	No. of animals	C_{max} (ng eq./mL)	$AUC_{0-\infty}$ (ng eq.·h/mL)	t_{max} (h)	$t_{1/2}$ (h)
Mice[a)]	Fasted	Blood	10	3/time point	119	–	0.25	–
		Plasma	10	3/time point	199	575	0.50	4.4[b)]
Male rats	Fasted	Blood	10	3	342.2 ± 49.1	1112.4 ± 199.6	0.33 ± 0.08	7.2 ± 2.5
	Fed		10	3	78.0 ± 12.7	725.7 ± 186.6	4.67 ± 1.76	2.8 ± 0.5
Female rats	Fasted		10	3	251.7 ± 8.0	808.9 ± 76.9	0.33 ± 0.08	6.2 ± 2.0
Dogs	Fasted	Blood	10	3	1594.6 ± 40.7	3813.8 ± 32.0[c)]	0.7 ± 0.2	4.0 ± 0.4

mean ± SE

a) Each parameter was calculated from the mean plasma concentration in 3 animals at each measuring time point.

b) Calculated from data obtained at 2, 4, and 6 hours after administration

c) AUC_{last}

3.(ii).A.(1).2) Repeated-dose administration study (4.2.3.2-3: Study 657[redacted]905)

Table 14 shows the pharmacokinetic parameters of unchanged acotiamide in the plasma following repeated oral administration of acotiamide (100-1000 mg/kg) once daily for 30 days to male and female rats.

Table 14. Pharmacokinetic parameters of unchanged acotiamide in the plasma following repeated oral administration of acotiamide for 30 days

Animal	Dose (mg/kg)	No. of animals	t_{max} (h)		C_{max} (ng/mL)		AUC_{0-24h} (ng·h/mL)	
			Day 1	Day 30	Day 1	Day 30	Day 1	Day 30
Male rats	100	3/time point	4.0	0.5	316.1	272.6	1972	1555
	300	3/time point	4.0	4.0	1395	978.0	7233	6743
	1000	3/time point	2.0	1.0	2410	1149	11,460	5575
Female rats	100	3/time point	4.0	1.0	240.1	550.5	2021	1970
	300	3/time point	4.0	0.5	1354	1374	8693	6279
	1000	3/time point	2.0	1.0	3495	2934	20,680	14,660

The parameters were calculated from the mean plasma concentration of 3 animals at each measuring time point.

3.(ii).A.(1).3) *In vitro* transcellular transport study (4.2.2.2-7: Study 622[redacted]001)

Membrane permeability of acotiamide (1, 5, 10, 50, 100, 500 μmol/L) was investigated using cells expressing human MDR1. The apparent permeability coefficient from the basolateral membrane side to the apicolateral membrane side "P_{app} (B-A)" was higher compared with that from the apicolateral membrane side to the basolateral membrane side "P_{app} (A-B)", with the P_{app} (B-A)/P_{app} (A-B) ratio being within the range from 8.1 to 14.4. The ratio tended to decrease with increasing acotiamide concentration, with K_m calculated to be 697 μmol/L.

Therefore, the applicant explained that the above results suggest that acotiamide serves as a substrate for P-glycoprotein (P-gp), but with only a low affinity.

3.(ii).A.(2) Distribution

3.(ii).A.(2).1) Tissue distribution following single oral dose (4.2.2.2-2, 4.2.2.3-1: Study 516[redacted]229, 611[redacted]003)

^{14}C-labeled acotiamide (10 mg/kg) was administered in a single oral dose to rats, and tissue radioactivity concentrations were measured at 0.5, 2, 6, 24, and 120 hours after administration. At 0.5 hours after administration, radioactivity was distributed widely in all the tissues studied, after which the plasma radioactivity concentration decreased over time. Radioactivity was higher than that observed in the plasma in the following tissues at the following time points: small intestine, stomach, urinary bladder, kidneys, liver, and artery at 0.5 hours after administration; stomach, kidneys, small intestine, liver, pancreas, brown fat, lung, urinary bladder, and large intestine at 2 hours after administration; and kidneys, small intestine, urinary bladder, liver, stomach, brown fat, pancreas, lung, large intestine, and submaxillary gland at 6 hours after administration. Radioactivity concentrations decreased below the lower limit of quantitation in all tissues at 24 hours after administration. Little or no radioactivity was distributed in the brain.

^{14}C-labeled acotiamide (10 mg/kg) was administered in a single oral dose to pigmented rats, and tissue radioactivity concentrations were measured at 0.5, 6, 24, 120, 240, and 360 hours after administration. Radioactivity was widely distributed in all the tissues studied at 0.5 hours after administration, with the radioactivity concentration in the kidneys and the liver being higher than that in the plasma. After that, the plasma radioactivity concentration decreased over time. The radioactivity concentration in the eyeballs was 12% of that in the plasma at 0.5 hours after administration and the concentration remained, albeit at a low level, suggesting the possibility that acotiamide was distributed in melanin-containing tissues. In contrast, no significant difference was observed in the changes over time in the radioactivity concentration in the white dermal tissue and that in the pigmented dermal tissue.

^{14}C-labeled acotiamide (10 mg/kg) was administered in a single oral dose to rats, and the radioactivity concentrations in organs and tissues were investigated by whole-body autoradiography up to 24 hours after administration. High radioactivity was detected in the gastrointestinal content, liver, kidneys, and stomach, among other organs, at 0.5 hours after administration, whereas, at 6 hours after administration, little or no radioactivity was detected except in the gastrointestinal content.

3.(ii).A.(2).2) Placental transfer in rats (4.2.2.3-2: Study 604[redacted]047)

^{14}C-labeled acotiamide (10 mg/kg) was administered in a single oral dose to pregnant rats on Gestation Day 19, and placental transfer of acotiamide at 0.5, 6, and 24 hours after administration was investigated. In maternal animals, radioactivity concentration in most tissues reached the maximum level at 0.5 hours after administration, whereas radioactivity concentration in the mammary gland reached the maximum level at 6 hours after administration. At 24 hours after administration, radioactivity in all tissues except the mammary gland and the placenta decreased below the plasma radioactivity concentration. In fetuses, radioactivity

concentration in most tissues reached the maximum level at 0.5 hours after administration, whereas radioactivity in the brain was detected only at 6 hours after administration.

Radioactivity concentration was ≤20 ng eq./mL in fetal blood, fetal tissues, amniotic fluid, and the whole fetal body.

3.(ii).A.(2).3 Distribution in blood cells

(a) *In vivo* studies

(4.2.2.2-1, 4.2.2.2-3, 4.2.2.2-4: Study 611[redacted]014, 604[redacted]028, 604[redacted]013)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to mice, blood/plasma radioactivity concentration ratio (Rb value) from 0.083 to 72 hours after administration was measured. As a result, Rb values at 0.5 hours and 2 hours after administration were 0.61 and 0.66, respectively. At 6 hours or more after administration, the blood radioactivity concentration was below the lower limit of quantitation. Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to rats, Rb values at 0.5, 2, and 6 hours after administration were 0.62, 0.70, and 1.02, respectively, showing a tendency of increasing over time.

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to dogs, the distribution of the radioactivity in blood cells[26] was measured from 0.25 to 48 hours after administration. The distribution rate reached the maximum level of 52.2% at 0.5 hours after administration, and then decreased over time to below the lower limit of quantitation by 24 hours after administration.

(b) *In vitro* studies (4.2.2.2-3, 4.2.2.3-3: Study 604[redacted]028, 611[redacted]001)

^{14}C-labeled acotiamide was added to the whole blood of rats, dogs, and humans to a final concentration of 0.14, 1.4, or 13 μg/mL, respectively. Rb values in rats, dogs and humans were 0.84 to 0.95, 1.21 to 1.29, and 0.85 to 0.95, respectively, showing no marked difference among different concentrations.

3.(ii).A.(2).4 Plasma protein binding

(a) *In vivo* studies (4.2.2.2-2, 4.2.2.3-5: Study 516[redacted]229, 604[redacted]024)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to rats and dogs, plasma protein binding rates were measured. The plasma protein binding rates at 0.5, 2, and 6 hours after administration were 39.37% to 57.21% in rats and 50.16% to 56.07% in dogs, showing almost constant value regardless of time after administration or blood radioactivity concentration.

(b) *In vitro* studies (4.2.2.2-2, 4.2.2.3-4: Study 516[redacted]229, 604[redacted]002)

^{14}C-labeled acotiamide was added to rat, dog, and human plasmas to a final concentration of 30 to 10,000 ng/mL and the plasma protein binding rate was measured. The plasma protein binding rates were 75.16% to 77.52%, 53.39% to 61.02%, and 84.21% to 85.95%, respectively. The binding rates with rat serum albumin, dog serum albumin, human serum albumin, and α_1-acid glycoprotein were 90.48% to 92.00%, 62.21% to 66.52%, 82.64% to 85.10%, and 9.56% to 13.91%, respectively.

3.(ii).A.(2).5 Distribution in gastric tissues

(a) Study on distribution in gastric tissues following subcutaneous administration (4.2.2.3-6: Study 622[redacted]009)

Following a single subcutaneous dose of acotiamide (10, 30, 100 mg/kg) to rats, concentrations of unchanged acotiamide in the plasma and in the gastric tissues, and the distribution rate of unchanged acotiamide in the gastric tissues to that in the plasma (Kp value) at 0.25 to 2 hours

[26] Rate of distribution in blood cells (%) = (100 – [100 – hematocrit value] / Rb value) × 100

after administration are shown in Table 15.

Table 15. Distribution rate in stomach following subcutaneous administration of acotiamide

Dose (mg/kg)	Time after administration (h)	Concentration in plasma (μg/mL)	Concentration in gastric tissue (μg/g of tissue)	Kp value of gastric tissue (mL/g of tissue)
10	0.25	3.83 ± 0.47	4.21 ± 1.22	1.1 ± 0.3
	0.5	3.26 ± 0.72	4.79 ± 0.86	1.5 ± 0.3
	1	1. 89 ± 0.41	4.05 ± 0.67	2.2 ± 0.5
	2	0.506 ± 0.109	2.98 ± 1.07	6.0 ± 2.0
30	0.25	10.4 ± 2.5	9.19 ± 2.56	0.89 ± 0.09
	0.5	9.65 ± 1.22	9.73 ± 1.80	1.0 ± 0.2
	1	6.91 ± 0.45	9.71 ± 1.02	1.4 ± 0.2
	2	1.80 ± 0.23	5.61 ± 0.86	3.1 ± 0.3
100	0.25	19.2 ± 5.5	14.4 ± 3.3	0.77 ± 0.11
	0.5	21.2 ± 3.5	21.6 ± 3.4	1.0 ± 0.1
	1	23.0 ± 2.4	24.3 ± 5.0	1.1 ± 0.3
	2	12.2 ± 1.8	15.3 ± 2.9	1.2 ± 0.1

n = 6, mean ± standard deviation (SD)

(b) Study on distribution in stomach following subcutaneous administration (4.2.2.3-9: Study 622[redacted]002)

Following a single subcutaneous administration of ^{14}C-labeled acotiamide (30 mg/kg) to rats, radioactivity distributions in the stomach and in the region around the nerve cells in the stomach at 0.5 and 1 hours after administration were investigated. After administration of acotiamide, radioactivity was rapidly distributed in the stomach, being detected in the body of the glandular stomach, pyloric antrum, and anterior stomach in the decreasing order of concentration. Radioactivity was also distributed in regions containing nerve cells in each site. In addition, using the stomach of acotiamide-untreated rats, the distribution of AChE activity was investigated using the active staining method. As a result, AChE activity was detected in the muscle layers, particularly in regions close to nerve cells.

(c) Study on distribution rate in gastric tissue under steady state (4.2.2.3-7: Study 622[redacted]011)

Following intravenous administration of acotiamide to fed rats under a constant injection rate of 2, 5, 10, 20, 50, 100 nmol/min/kg, the concentrations of unchanged acotiamide in the plasma, gastric tissue, and skeletal muscle were measured. Total-body clearance (mean ± standard deviation [SD]) was 34.1 ± 6.1 to 38.7 ± 3.9 mL/min/kg, demonstrating that there was no change in the clearance within this dose range. Kp value (mean ± SD) was 2.4 ± 0.3 to 4.1 ± 0.3 mL/g of tissue with the gastric tissue and 0.71 ± 0.17 to 0.85 ± 0.09 mL/g of tissue with the skeletal muscle, showing that Kp value of the gastric tissue was 3.2- to 5.8-fold that of the skeletal muscle. Kp value of the skeletal muscle was constant regardless of plasma acotiamide concentration, whereas that of the gastric tissue became saturated with increasing plasma acotiamide concentration.

(d) Study on the clearance after initial uptake into gastric tissue (4.2.2.3-10: Study 622[redacted]002)

Following a single intravenous dose of ^{14}C-labeled acotiamide (0.1, 0.5, 1, 5 mg/kg) to rats, clearances of initial uptake ($CL_{inf, app}$) into the stomach, skeletal muscle, liver, kidneys, and brain tissues were investigated using integration plot analysis.

$CL_{inf, app}$ of radioactivity was highest in the kidneys, followed in descending order by the liver, stomach, skeletal muscle, and brain. In the stomach, $CL_{inf, app}$ of acotiamide-derived radioactivity was higher than that of the radioactivity derived from ^{3}H-inulin which was added

simultaneously as an extracellular marker. In the skeletal muscle, in contrast, $CL_{inf, app}$ of acotiamide-derived radioactivity was similar to that of inulin-derived radioactivity and, in the brain, $CL_{inf, app}$ of acotiamide-derived radioactivity was comparable to, or less than, that of inulin-derived radioactivity. $CL_{inf, app}$ values of the liver and the kidneys, organs that are involved in the elimination process of drugs, were higher than those in other tissues measured. In the stomach, liver, and kidneys, $CL_{inf, app}$ of acotiamide-derived radioactivity decreased with increasing dose of acotiamide, showing saturability.

(e) Study on distribution in gastric tissue following intraduodenal administration (4.2.2.3-8: Study 622[redacted]001)

Following a single intraduodenal dose of ^{14}C-labeled acotiamide (30 mg/kg) to dogs, radioactivity concentrations in the blood, plasma, and gastric tissue were measured at 0.5, 1, 2, and 4 hours after administration by a quantitative autoradiography. The blood radioactivity concentration reached the maximum level of 2117 ng eq./mL at 0.5 hours after administration, after which it decreased over time to 512 ng eq./mL by 4 hours after administration. The plasma radioactivity concentration changed over time in a similar manner as that in the blood. In the gastric tissues, the radioactivity concentrations in the gastric fundus, in the gastric body, and in the gastric antrum were similar. The radioactivity concentrations in the gastric fundus, body, and antrum reached the maximum level of 643 to 835 ng eq./g at 0.5 hours after administration, after which it decreased over time to the range of 201 to 272 ng eq./g by 4 hours after administration.

3.(ii).A.(3) Metabolism

3.(ii).A.(3).1 Study on *in vitro* metabolism (4.2.2.4-7: Study 611[redacted]024)

^{14}C-labeled acotiamide (10 μmol/L) was incubated in liver microsomes of humans, dogs, rabbits, SD rats, Fischer rats, and mice. Unchanged acotiamide was mainly detected, and metabolites were detected in the liver microsomes of all animal species except dogs. The structures of the following metabolites were identified: M-1 which is a glucuronide conjugate of unchanged acotiamide, and M-4 which is a deisopropyl form of unchanged acotiamide. The percentages of unchanged acotiamide and of the metabolites with identified structure at 60 minutes after the start of reaction are shown in Table 16.

Table 16. *In vitro* metabolites of acotiamide[a)]

	Unchanged acotiamide	M-1	M-4
Humans	42.3%	48.5%	4.0%
Dogs	100.0%	–	–
Rabbits	53.9%	33.9%	3.0%
SD rats	90.3%	9.7%	–
Fischer rats	95.3%	4.7%	–
Mice	61.1%	21.4%	2.8%

a) Percentage of the peak area of unchanged acotiamide and each metabolite to the total peak area in radiochromatogram

3.(ii).A.(3).2 Study on metabolites in plasma, urine, feces, and bile of mice (4.2.2.2-1: Study 611[redacted]014)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to mice, metabolite profiles were investigated in plasma at 0.5, 2, and 4 hours after administration, in urine during 0 to 6 hours and 6 to 24 hours after administration, and in feces up to 24 hours after administration.

In the plasma, unchanged acotiamide and 3 metabolites were mainly detected. Of the metabolites detected, those with identified structures were M-1, and M-2 which is a glucuronide conjugate of deisopropyl acotiamide. The percentages of the radioactivity of unchanged acotiamide and each metabolite in the plasma at 0.5 hours after administration to the total

plasma radioactivity were 13.4% for the unchanged acotiamide, 55.9% for M-1, and 3.9% for M-2. Both unchanged acotiamide and the metabolites decreased over time, with the radioactivity detected after 4 hours being 10.5% for unchanged acotiamide and 33.7% for M-1 and none for M-2. In the urine, unchanged acotiamide and 3 metabolites were mainly detected. Of the metabolites detected, those with identified structure were M-1 and M-2. The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite to the radioactivity administered were 0.3% ± 0.3% for unchanged acotiamide, 1.1% ± 0.5% for M-1, and 0.1% ± 0.1% for M-2 in the urine pooled from 0 to 6 hours after administration; and 0.6% ± 0.9% for unchanged acotiamide, 1.0% ± 0.8% for M-1, and below the lower limit of quantitation for M-2 in the urine pooled from 6 to 24 hours after administration. In the feces, little or no metabolites were detected, and the percentage (mean ± SD) of the radioactivity of unchanged acotiamide in the feces to the radioactivity administered was 78.3% ± 15.2%.

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to bile duct-cannulated mice, the metabolite profiles in the bile was studied from 0 to 6 hours, and from 6 to 24 hours, after administration. Unchanged acotiamide was not detected, and mainly 3 metabolites were detected. Of the metabolites detected, those with identified structure were M-1 and M-2. The percentage (mean ± SD) of the radioactivity of each metabolite to the radioactivity administered was 8.1% ± 1.2% for M-1 and 0.3% ± 0.2% for M-2 in the bile collected from 0 to 6 hours administration; and 3.3% ± 1.5% for M-1 and 0.2% ± 0.2% for M-2 in the bile collected from 6 to 24 hours after administration.

3.(ii).A.(3).3) Studies on metabolites in plasma, urine, feces, and bile of rats (4.2.2.2-2, 4.2.2.2-3, 4.2.2.4-1: Study 516[redacted]229, 604[redacted]028, 604[redacted]017)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to rats, metabolite profiles were investigated in plasma at 0.5, 2, and 6 hours after administration, and in urine and feces up to 24 hours after administration.

The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the plasma at 0.5 hours after administration to the total plasma radioactivity were 17.70% ± 2.31% for unchanged acotiamide, 70.09% ± 2.76% for M-1, 2.84% ± 0.45% for M-2, and 0.76% ± 0.54% for M-4. No radioactivity was detected for M-4 at 2 hours after administration and for M-2 at 6 hours after administration. The percentage (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the urine to the total urinary radioactivity was 20.63% ± 1.58% for unchanged acotiamide, 57.68% ± 1.69% for M-1, and 4.09% ± 0.40% for M-2. M-4 was not detected. The percentage (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the feces to the total fecal radioactivity was 82.41% ± 8.46% for unchanged acotiamide, 0.33% ± 0.33% for M-1, 0.23% ± 0.23% for M-2, and 11.28% ± 5.94% for M-4.

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to bile duct-cannulated rats, metabolite profile in the bile collected up to 8 hours after administration was studied. The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the bile to the total biliary radioactivity were 6.32% ± 0.94% for unchanged acotiamide, 76.79% ± 2.43% for M-1, 12.06% ± 1.77% for M-2, and 0.50% ± 0.12% for M-4.

3.(ii).A.(3).4) Studies on metabolites in plasma, urine, feces, and bile of dogs (4.2.2.3-5, 4.2.2.4-2–4.2.2.4-4: Study 604[redacted]024, 604[redacted]046, 604[redacted]010, 604[redacted]057)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to dogs, metabolite profiles were investigated in plasma at 0.5, 2, and 6 hours after administration, and in urine and feces collected up to 24 hours after administration.

The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite

in the plasma at 0.5 hours after administration to the total plasma radioactivity were 74.22% ± 5.13% for unchanged acotiamide, 12.75% ± 0.66% for M-1, 1.54% ± 0.98% for M-2, and 0.56% ± 0.29% for M-4. No radioactivity was detected for M-4 at 6 hours after administration. The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the urine to the total urinary radioactivity were 62.80% ± 3.19% for unchanged acotiamide, 20.18% ± 1.10% for M-1, 5.40% ± 1.15% for M-2, and 2.67% ± 0.40% for M-4. The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the feces to the total fecal radioactivity were 90.08% ± 0.49% for unchanged acotiamide, 0.21% ± 0.11% for M-1, 0.29% ± 0.14% for M-2, and 5.67% ± 0.41% for M-4.

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to bile duct-cannulated dogs, metabolite profile in the bile collected up to 24 hours after administration was studied. The percentages (mean ± SD) of the radioactivity of unchanged acotiamide and each metabolite in the bile to the total biliary radioactivity were 13.68% ± 5.42% for unchanged acotiamide, 65.05% ± 3.61% for M-1, 8.77% ± 2.35% for M-2, and 3.18% ± 0.67% for M-4.

3.(ii).A.(3).5) Studies on metabolites in plasma, urine, and feces of humans (4.2.2.4-5, 4.2.2.4-6: Study 622[redacted]014, 611[redacted]001)

Following a single oral dose of a mixed solution of ^{14}C-labeled and unlabeled acotiamide to healthy adult male subjects, metabolite profiles were investigated in plasma at 0.5, 1, 1.5, 2, and 4 hours after administration, in the urine up to 72 hours after administration, and in the feces up to 120 hours after administration.

In the plasma, unchanged acotiamide and metabolites M-2 and M-1 were detected. In the urine, M-2 and M-1 were present at 0.66% ± 0.27% (mean ± SD) and 3.63% ± 0.97%, respectively, of the dose administered. In the feces, unchanged acotiamide, M-1, M-4, and M-2 were present at 66.85% ± 17.59%, 4.49% ± 6.00%, 3.73% ± 1.56%, and 0.59% ± 0.61%, respectively, of the dose administered.

3.(ii).A.(3).6) Study on CYP isoforms involved in metabolism (4.2.2.4-8: Study 611[redacted]004)

Acotiamide (10, 100 μmol/L) was incubated with microsomes expressing human CYP isoforms (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5, CYP4A11). Table 17 shows the concentration of M-4 (deisopropyl acotiamide) formed. The highest amount of M-4 was formed when acotiamide was incubated with CYP2C8, and a slight amount was also formed when incubated with CYP1A1, CYP3A4, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP3A5, or CYP4A11.

Table 17. Concentration of M-4 (deisopropyl acotiamide) formed

CYP isoform	Acotiamide concentration (mol/L)	
	10	100
CYP1A1	263.84 ± 3.31	1215.05 ± 35.84
CYP1A2	–	–
CYP2A6	–	–
CYP2B6	–	–
CYP2C8	1390.76 ± 75.84	8830.10 ± 369.73
CYP2C9	–	163.28 ± 4.63
CYP2C18	–	38.36 ± 2.94
CYP2C19	–	100.13 ± 5.33
CYP2D6	–	139.89 ± 6.20
CYP2E1	–	–
CYP3A4	203.24 ± 9.39	1085.92 ± 34.72
CYP3A5	–	139.67 ± 3.21
CYP4A11	–	39.88 ± 7.54

n = 3, mean ± SD

3.(ii).A.(3).7) Study on UGT isoforms involved in metabolism (4.2.2.1-6, 4.2.2.4-9: Study 622[redacted]048, 622[redacted]017)

Acotiamide (1 μmol/L) was incubated with microsomes expressing human UGT isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9, UGT1A10, UGT2B7, UGT2B15). Unchanged acotiamide was metabolized mainly by UGT1A8 and UGT1A9, and only slightly by UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A10, UGT2B7, and UGT2B15. K_m of acotiamide for UGT1A8 and UGT1A9 was calculated to be 1426.51 and 43.13 μmol/L, respectively.

3.(ii).A.(3).8) Study on enzyme induction by acotiamide (4.2.2.4-10: Study 604[redacted]055)

Following repeated oral administration of acotiamide (10, 100, 1000 mg/kg) once daily for 7 days to rats, protein concentration in liver microsomes, contents of cytochrome P450 and b5, and activities of the following enzymes were measured: aniline hydroxylase, aminopyrine *N*-demethylase, 7-ethoxycoumarin *O*-deethylase, NADPH-cytochrome c reductase, and *p*-nitrophenyl-glucuronidase. All values were similar to those observed in the control group, which suggested that acotiamide did not have any enzyme-inducing activities.

3.(ii).A.(4) Excretion

3.(ii).A.(4).1) Urinary, fecal, and biliary excretion (4.2.2.2-1, 4.2.2.2-2, 4.2.2.2-4: Study 611[redacted]014, 516[redacted]229, 604[redacted]013)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to mice, rats, and dogs, urinary and fecal excretion rates of radioactivity to the total radioactivity administered were investigated. Table 18 shows the results.

Table 18. Urinary and fecal excretion rate of radioactivity following a single oral dose of ^{14}C-labeled acotiamide

Animal species	Time (h)	Urinary excretion rate (%)	Fecal excretion rate (%)
Mice[a)]	0-24	6.0 ± 5.6	80.1 ± 13.3
	0-120	9.0 ± 7.3	89.2 ± 10.4
Rats	0-24	7.23 ± 1.32	68.74 ± 10.09
	0-120	7.42 ± 1.29	91.30 ± 1.49
Dogs	0-24	6.6 ± 0.6	63.9 ± 15.9
	0-168	7.0 ± 0.6	93.0 ± 1.5

n = 3, mean ± SE
a) Mean ± SD

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to bile duct-cannulated mice, rats, and dogs, urinary, fecal, and biliary excretion rates of radioactivity relative to the total radioactivity administered were investigated. Table 19 shows the results.

Table 19. Urinary, fecal, and biliary excretion rates following a single oral dose of ^{14}C-labeled acotiamide

Animal species	Time (h)	Urinary excretion rate (%)	Fecal excretion rate (%)	Biliary excretion rate (%)
Mice[a)]	0-24	5.2 ± 4.9	87.6 [b)]	12.0 ± 0.8
Rats	0-48	24.41 ± 1.14	35.47 ± 4.91	37.16 ± 2.45
Dogs	0-48	10.3 ± 1.9	61.6 ± 1.5	20.2 ± 2.7

n = 3, mean ± SE
a) Mean ± SD
b) Value observed in 1 animal

3.(ii).A.(4).2) Enterohepatic circulation in rats (4.2.2.2-2: Study 516[redacted]229)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to rats, bile collected up to 2 hours after administration was administered intraduodenally to a different set of rats. As a result, the urinary, fecal, and biliary excretion rates (mean ± SE) of radioactivity up to 48 hours after

administration were 3.47% ± 0.29%, 90.84% ± 4.29%, and 3.82 ± 0.13%, respectively. From urinary and biliary excretion rates of radioactivity, the reabsorption rate of biliary excreted radioactivity was calculated to be approximately 7%.

3.(ii).A.(4).3) Excretion into breast milk (4.2.2.3-2: Study 604[redacted]047)

Following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) to female rats 13 days after delivery, radioactivity in plasma and breast milk was measured at 0.5, 2, 4, 8, 24, and 48 hours after administration. Radioactivity in plasma and in breast milk reached the maximum level (1113.2 ± 258.5 and 856.4 ± 214.9 ng·eq/mL, respectively, [mean ± SE]) at 0.5 and 2 hours, respectively, after administration, after which it decreased, falling below the lower limit of quantitation at 24 hours after administration in both samples. The ratio of radioactivity concentration in breast milk to that in plasma reached the maximum level (6.05 ± 1.50 [mean ± SE]) at 4 hours after administration.

3.(ii).A.(5) Pharmacokinetic drug-drug interactions

3.(ii).A.(5).1) *In vitro* CYP inhibition (4.2.2.6-1, 4.2.2.6-2: Study 622[redacted]002, 611[redacted]037)

Effect of acotiamide on CYP isoforms (CYP1A1/2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4) was investigated using human liver microsomes and CYP2C8-expressing microsomes. The inhibition constant of acotiamide against enzymatic activity of CYP isoforms was 60.7 μmol/L for CYP2A6 and ≥100 μmol/L for all other isoforms, showing no strong inhibitory activities.

3.(ii).A.(5).2) *In vitro* P-gp inhibition (4.2.2.6-3: Study 622[redacted]052)

Effect of acotiamide (0.5, 5, 50 μmol/L) on transcellular transport of ^{3}H-labeled digoxin, a substrate for P-gp, was investigated using Caco-2 cells. P_{app} (B-A) of ^{3}H-labeled digoxin decreased by 11.1% to 21.1%, showing a weak inhibition, but no dose dependency was observed.

3.(ii).B Outline of the review by PMDA

3.(ii).B.(1) Transfer and distribution of acotiamide in gastric tissue

Based on the results of the studies on the transfer and distribution of acotiamide in the gastric tissue following the administration, the applicant discussed as follows: (1) After subcutaneous administration of acotiamide, unchanged acotiamide is transferred to the gastric tissue in a saturable manner, (2) the distribution of radioactivity after subcutaneous administration of ^{14}C-labeled acotiamide overlaps the distribution of AChE activity, (3) unchanged acotiamide is distributed in higher concentration in the gastric smooth muscles than in the skeletal muscles, and (4) the clearance of initial uptake in the gastric tissue following intravenous administration of ^{14}C-labeled acotiamide and the distribution in the gastric tissue at steady state during intravenous administration of unlabeled acotiamide at a constant rate are saturable, suggesting that a carrier may be involved in the uptake process into the gastric tissue.

PMDA considers as follows:

Since these studies were not conducted by oral administration, which is the administration route planned for clinical use, it is difficult to conclude whether the above results are applicable in clinical settings. However, based on the discussion on the transfer to the gastric tissue following subcutaneous or intravenous administration of acotiamide and on the distribution of the radioactivity in the gastric tissue following intraduodenal administration of ^{14}C-labeled acotiamide, the study results suggest the transfer of acotiamide from the blood to the gastric tissue in rats, although the mechanism of the transfer remains unclear.

3.(ii).B.(2) Melanin affinity

PMDA, by pointing out the finding that acotiamide-derived radioactivity tended to remain in the

eyeballs for a relatively long period of time, asked the applicant to explain the possibility that acotiamide may affect melanin-rich tissues such as the eyeballs and the skin. PMDA also asked the applicant to explain the photo-safety based on the safety information obtained from the toxicity studies and clinical studies.

The applicant explained as follows:
The effect of acotiamide on the eyeballs and the skin was investigated from the following 2 aspects: (1) the effect on the eyeballs caused by AChE inhibition, the mechanism of action of acotiamide, and (2) the effect on the eyeballs and the skin caused by other mechanisms.

Regarding the effect on the eyeballs caused by AChE inhibition, miosis was not observed at 100 mg/kg in any animal species studied in toxicological studies;[27] the symptom was observed only in the ≥300 mg/kg dose groups [see "3.(iii).A.(2) Repeated-dose toxicity" and "3.(iii).A.(5) Reproductive and developmental toxicity"]. When multiple doses of acotiamide were orally administered to healthy adult male subjects three times daily at the recommended clinical dose (100 mg), the plasma concentration of unchanged acotiamide was 109.0 ng/mL,[28] while the plasma concentration of unchanged acotiamide observed following administration in rats or dogs at 100 mg/kg, the dose at which no miosis was observed, was approximately 3- to 37-fold that in humans. Except for miosis, no AChE-associated toxicological findings were observed in rats. In the 3-month repeated oral dose toxicity study in dogs, lacrimation, exposure of nictating membrane, and hyperemia of bulbar conjunctiva and episclera were observed at 300 mg/kg and, in the 9-month repeated oral dose toxicity study, lacrimation was observed at 100 mg/kg. However, the plasma concentration of unchanged acotiamide in dogs at 30 mg/kg, the dose that did not show any toxicological findings in dogs, was approximately 10- to 33-fold that in humans. In addition, in the Japanese clinical studies in FD patients, there were no AChE inhibition-induced eyeball-related adverse events for which causal relationship with acotiamide was suspected.

Regarding effects other than AChE inhibition, in the 4-week repeated oral dose toxicity study in rats (4.2.3.2-3) and in the 4-week repeated oral dose toxicity study in dogs (4.2.3.2-5), ophthalmological examination showed no findings suggestive of ocular disorder, nor were any dermal toxicity findings noted. As for the effect in humans, the Japanese clinical studies in FD patients did not show any ocular adverse events for which causal relationship with acotiamide was suspected. In contrast, the incidence of adverse events related to skin and subcutaneous tissue disorders was slightly higher in the acotiamide groups (2.5%, 38 of 1530 subjects) compared with the placebo group (0.6%, 4 of 714 subjects). However, tabulation of adverse events related to skin and subcutaneous tissue disorders in the acotiamide group by severity showed that most of the adverse events were minimal, and there were no specific events that occurred at a particularly higher frequency than others.

The above results suggest that acotiamide is unlikely to affect melanin-rich tissues such as the eyeballs and the skin.

Although the UV spectrum of acotiamide has an absorption maximum above 280 nm, acotiamide is photostable [see "2.A.(2).4) Stability of drug product"]. In addition, no findings suggestive of phototoxicity were observed in the clinical or nonclinical studies.

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[27] Four-week repeated oral dose toxicity study in rats (4.2.3.2-3), study of fertility and early embryonic development to implantation in rats (4.2.3.5.1-1), and 4-week repeated oral dose toxicity study in dogs (4.2.3.2-5)

[28] The maximum plasma concentration of unchanged acotiamide during the multiple administration in the phase I multiple dose study ([redacted]) (duration of multiple administration, 7 days)

PMDA considers as follows:
In pigmented rats, acotiamide-derived radioactivity tended to remain distributed in the eyeballs for a relatively long period of time, but no eye-related adverse events were observed in the clinical studies. Although the incidence of adverse events related to the skin and subcutaneous tissue disorders was slightly higher in the acotiamide groups compared with the placebo group, most of them were mild in severity and there was no tendency of any specific event occurring at a high frequency. On the basis of the above results, PMDA accepted the applicant's explanation that acotiamide is unlikely to affect melanin-rich tissues such as the eyeballs and the skin.

3.(iii) Summary of toxicology studies

3.(iii).A Summary of the submitted data

Toxicology studies of acotiamide conducted include single-dose toxicity, repeated-dose toxicity, genotoxicity, carcinogenicity, reproductive and developmental toxicity, and other toxicity studies (antigenicity studies, studies on uterine enlargement).

Both male and female animals were used unless otherwise specified. The vehicle used was 0.5 w/v% MC solution in rats, mice, and rabbits, and gelatin capsules in dogs. In all studies, acotiamide doses are expressed in terms of the salt, while the plasma concentration of acotiamide hydrochloride hydrate is expressed in terms of acotiamide.

3.(iii).A.(1) Single- dose toxicity (4.2.3.1-2, 4.2.3.1-3: Study 657[redacted]904, 514[redacted]124)

The vehicle or acotiamide (500, 1000, 2000 mg/kg) was administered to rats, and acotiamide (60, 200, 600, 2000 mg/kg) to male dogs, in a single oral dose. No death occurred at any dose, from which the approximate lethal dose was determined to be >2000 mg/kg in both rats and male dogs. Major toxicological findings were miosis in rats, miosis, vomiting/vomiting-like behavior, loose stools, decreased locomotor activity, and twitching in dogs. All findings were determined to be due to the pharmacological action of acotiamide.

3.(iii).A.(2) Repeated-dose toxicity

Repeated-dose toxicity studies were conducted by oral administration both in rats (4 weeks, 6 months) and in dogs (4 weeks, 3 months, 9 months). Major acotiamide-induced toxicological findings observed in the repeated-dose studies were miosis in both male and female rats, and miosis, lacrimation, salivation, tremor, abnormal gait, decreased locomotor activity, hyperemia of bulbar conjunctiva and episclera, exposure of nictating membrane, vomiting/vomiting-like behavior, and increased total cholesterol in both male and female dogs, and increased phospholipids in male dogs. Unless otherwise specified, findings were observed in both male and female animals.

3.(iii).A.(2).1) Four-week repeated-dose oral toxicity study in rats (4.2.3.2-3: Study 657[redacted]905)

The vehicle or acotiamide (100, 300, 1000 mg/kg/day) was administered orally to rats for 4 weeks. No death occurred in any of the treatment groups. Miosis was observed in the ≥300 mg/kg/day dose groups throughout the treatment period. Acotiamide had no other clear effects. The no observed adverse effect level (NOAEL) is determined to be 100 mg/kg/day in both male and female rats.

3.(iii).A.(2).2) Six-month repeated-dose oral toxicity study in rats (4.2.3.2-4: Study 603[redacted]067)

The vehicle or acotiamide (10, 30, 100, 300 mg/kg/day) was administered orally to rats for 6 months. The control group and the 300 mg/kg/day group included a recovery group with 1-month washout period. No death occurred in any of the groups. No toxicologically significant effect was noted in clinical observations, body weight, food consumption, histopathology, etc. The NOAEL is determined to be 300 mg/kg/day in both male and female rats.

3.(iii).A.(2).3) Four-week repeated-dose oral toxicity study in dogs (4.2.3.2-5: Study 514[redacted]133)

The vehicle or acotiamide (100, 300, 1000 mg/kg/day) was administered orally to dogs for 4 weeks. No death occurred in any of the treatment groups. In the ≥300 mg/kg/day dose groups, the following adverse events were observed: salivation in males; and miosis, hyperemia of bulbar conjunctiva and episclera, and increased total cholesterol in females. In the 1000 mg/kg/day group, the following adverse events were observed: exposure of nictating membrane, palpebral edema, decreased locomotor activity, abnormal gait, tremor, and vomiting/vomiting-like behavior in both males and females; miosis, hyperemia of bulbar conjunctiva and episclera, increased total cholesterol and phospholipids in males, and salivation in females. The NOAEL is determined to be 100 mg/kg/day in both male and female dogs.

3.(iii).A.(2).4) Three-month repeated-dose oral toxicity study in dogs (4.2.3.2-6: Study 603[redacted]059)

The vehicle or acotiamide (30, 100, 300 mg/kg/day) was administered orally to dogs for 3 months. The control group and the 300 mg/kg/day group included a recovery group with 1-month washout period. No death occurred in any of the treatment groups. In the 300 mg/kg/day group, lacrimation and salivation were observed in both males and females, and exposure of nictating membrane and hyperemia of bulbar conjunctiva and episclera were observed in males. The NOAEL is determined to be 100 mg/kg/day in both male and female dogs.

3.(iii).A.(2).5) Nine-month repeated-dose oral toxicity study in dogs (4.2.3.2-7: Study 621[redacted]071)

The vehicle or acotiamide (30, 100, 300 mg/kg/day) was administered orally to dogs for 9 months. No death occurred in any of the treatment groups. In the ≥100 mg/kg/day dose groups, salivation was observed in males. In the 300 mg/kg/day group, lacrimation was observed in males and salivation in females. The NOAEL is determined to be 30 mg/kg/day in males and 100 mg/kg/day in females.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1-2–4, 4.2.3.3.2-3, 4.2.3.3.2-4: Study 621[redacted]077, 514[redacted]135, 611[redacted]542, 657[redacted]906, 603[redacted]069)

As genotoxicity studies, bacterial reverse mutation tests, chromosomal aberration tests with cultured Chinese hamster-derived lung fibroblast cell line (CHL) and with human peripheral lymphocytes were performed as *in vitro* studies, and bone marrow micronucleus tests in rats after oral administration and unscheduled DNA synthesis (UDS) tests in rat liver cells as *in vivo* studies.

The *in vitro* chromosomal aberration test with CHL showed a tendency of an increase in cells with structural chromosome aberrations at ≥2600 μg/mL (the concentration inhibiting the cell growth by ≥50%) in the presence of S9 mix. However, in the chromosomal aberration test with human peripheral lymphocyte and the UDS test with rat liver cells which were conducted as additional tests, negative results were obtained. In addition, in the micronucleus tests in rats, there was no significant increase in the number of juvenile cells containing micronuclei at the

dose of 2000 mg/kg/day.[29] On the basis of these results, the applicant has determined that acotiamide is negative for genotoxicity.

3.(iii).A.(4) Carcinogenicity

As preliminary tests for carcinogenicity tests, a 4-week oral administration study in mice and a 13-week oral administration study in rats were conducted, after which 24-month carcinogenicity studies in mice and rats were conducted. No acotiamide-induced neoplastic lesion was observed in mice. In rats, in contrast, endometrial adenocarcinomas were observed in the acotiamide group at a frequency exceeding the laboratory's historical data during the study period. On the basis of these results, "a 2-step uterine carcinogenicity study in rasH2 mice" was conducted using the mouse strain induced to develop endometrial adenocarcinoma by treatment with *N*-nitroso-*N*-ethylurea (ENU), in order to investigate the effect of acotiamide to modify the occurrence of endometrial adenocarcinoma. The mouse model used in the study was the rasH2 mouse that can be induced to develop endometrial adenocarcinoma at the highest frequency in a relatively short period of time at the current scientific level. As a result, acotiamide did not have any effect on the incidence of endometrial adenocarcinoma or on the number of atypical hyperplasia per animal, from which it was determined that acotiamide had no effect on ENU-induced uterine carcinogenesis in rasH2 mice.

3.(iii).A.(4).1) Carcinogenicity in mice

(a) Four-week preliminary carcinogenicity study in mice (4.2.3.4.1-1: Study 621[redacted]057)

The vehicle or acotiamide (1000, 1500, 2000 mg/kg/day) was administered orally for 4 weeks to B6C3F1 mice (n = 5/sex/dose). No death occurred. Females in the 2000 mg/kg/day group showed increased ovary weight and a dose-dependent increase in relative ovary weight. The toxicokinetic (TK) study showed that, in the maximum dose group (2000 mg/kg/day), C_{max} (11,996.6-18,065.6 ng/mL) and $AUC_{0\text{-}24h}$ (14,698.8-20,445.6 ng·h/mL) were ≥100- and 23-fold, respectively, higher than C_{max} and AUC_{last} achieved at the recommended clinical dose.

On the basis of the above results, the applicant considered it appropriate to set the maximum dose at 2000 mg/kg/day in the 13-week preliminary study on carcinogenicity in mice.

(b) Thirteen-week preliminary carcinogenicity study in mice (4.2.3.4.1-2: Study 621[redacted]059)

The vehicle or acotiamide (1000, 1500, 2000 mg/kg/day) was administered orally for 13 weeks to B6C3F1 mice (n = 10/sex/dose). No death occurred. Females of the 2000 mg/kg/day group showed an increase in the relative weight of the heart, but the histopathological examination did not reveal any effect of acotiamide. The TK study showed that, in the maximum dose group (2000 mg/kg/day), C_{max} (9706.9-12,984.5 ng/mL) and $AUC_{0\text{-}24h}$ (10,671.0-19,516.7 ng·h/mL) were ≥89- and 17-fold, respectively, higher than C_{max} and AUC_{last} achieved at the recommended clinical dose.

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[29] In the single oral dose toxicity study in rats (4.2.3.1-2: Study 657[redacted]904), no death was observed either in males or in females at the dose of 2000 mg/kg/day. This dose was therefore used as the maximum dose. In the micronucleus test in rats, C_{max} (2448 ng/mL) and AUC (30,550 ng·h/mL, the sum of AUC on Day 1 and Day 2) in the 2000 mg/kg/day group were ≥22 and ≥49 times, respectively, the C_{max} (109.0 ng/mL) and AUC_{last} (621.9 ng·h/mL) achieved following the administration of the recommended clinical dose in humans. Therefore, the dose was determined appropriate as the dose used for the maximum exposure.

For details of C_{max} and AUC_{last} in the recommended clinical dose, see the following descriptions.

C_{max} Maximum plasma concentration of unchanged acotiamide during the multiple administration period in the phase I multiple dose study ([redacted]) (duration of multiple administration: 7 days) in which acotiamide 100 mg was administered orally three times daily to healthy adult male subjects.

AUC_{last} The value obtained by tripling the AUC_{last} (207.3 ng·h/mL) after the last dose (single dose administration on the last day) in the phase I multiple dose study ([redacted]) (duration of multiple administration: 7 days)

In all comparisons with the "recommended clinical dose" described below, these values are used for comparison.

On the basis of the above results, the applicant considered it appropriate to set the maximum dose at 2000 mg/kg/day in the carcinogenicity study in mice.

(c) Twenty-four-month carcinogenicity study in mice (4.2.3.4.1-3: Study 621[redacted]053)

The vehicle or acotiamide (200, 600, 2000 mg/kg/day) was administered orally for 24 months to B6C3F1 mice[30] (n = 50/sex/dose). No acotiamide-related neoplastic lesion was observed.

As the neoplastic lesion, uterine histiocytic sarcoma was observed in 0 of 50 animals (0%) in the vehicle group, 4 of 50 animals (8%) in the 200 mg/kg/day group, 5 of 50 animals (10%) in the 600 mg/kg/day group, and 5 of 50 animals (10%) in the 2000 mg/kg/day group, with the increase being statistically significant in the ≥600 mg/kg/day dose groups. According to the historical data accumulated (from 5 studies) in the different laboratory that used B6C3F1 mice supplied by the same supplier, which was published in 1997 (*Annual report of Hatano Research Institute*, 1997;20: 7-19), the mean incidence of uterine histiocytic sarcomas was 3.5%, ranging from 0% to 12% depending on the batch of animals. In contrast, according to the historical data from the studies ([redacted] studies) conducted during the same period (20[redacted] to 20[redacted]), and in the same laboratory, in which the 24-month carcinogenicity study in mice was conducted, the mean incidence of uterine histiocytic sarcomas in B6C3F1 mice supplied by the same supplier was [redacted]%, with a maximum of [redacted]%. Also, according to the results of the survey of the incidence of histiocytic sarcoma in the uterus and in other organs and tissues that occurred in studies[31] conducted in the same study site in 20[redacted], in addition to the historical data of these [redacted] studies, the mean incidence of histiocytic sarcoma was as high as [redacted]% (range: [redacted]%-[redacted]%), among which the incidence in the uterus was [redacted]% (range: [redacted]%-[redacted]%). In addition, according to the survey on the incidence of histiocytic sarcoma in recent carcinogenicity studies ([redacted] studies) which were conducted recently in the same study site using B6C3F1 mice supplied by a different supplier[32] to examine the possibility that the site of histiocytic sarcoma may differ depending on bleeder, the incidence in the uterus among all histiocytic sarcomas was [redacted]% (range: [redacted]%-[redacted]%), which confirmed that histiocytic sarcoma occurs frequently in the uterus in B6C3F1 mice. The incidence of uterine histiocytic sarcomas observed in the acotiamide group was within the range of the laboratory's historical data. Other histologies and biological behaviors (timing of occurrence, frequency of metastasis, histology of metastatic site) were not different from those of spontaneous uterine histiocytic sarcoma, and the sarcoma did not occur earlier than the spontaneous one and there was no multi-organ occurrence. On the basis of these findings, the applicant determined that uterine histiocytic sarcoma observed in the acotiamide group is a spontaneous disease.

From these results, the applicant has considered that acotiamide is not carcinogenic in mice.

3.(iii).A.(4).2) Carcinogenicity in rats

(a) Four-week preliminary carcinogenicity study in rats (4.2.3.4.1-4: Study 621[redacted]058)

The vehicle or acotiamide (1000, 1500, 2000 mg/kg/day) was administered orally to F344 rats (n = 5/sex/dose) for 4 weeks. No death occurred. Animals in the acotiamide groups showed miosis and loose stools which were probably due to the pharmacological action of acotiamide. Males in all acotiamide groups showed increased total cholesterol level. Males and females at ≥1500 mg/kg/day showed a decrease in the relative weight of the spleen. Males in the 2000 mg/kg/day group showed a decrease in the absolute weight of the spleen, while females in the same dose group showed decreases in erythrocytic parameters and an increase in the absolute weight of the ovary. The TK study showed that, in the maximum dose group (2000 mg/kg/day),

[30] Supplier: Charles River Laboratories Japan, Inc.

[31] Studies on test substances that neither have any effect on hormone levels or on uterus nor increase the incidence of histiocytic sarcoma were selected.

[32] Japan SLC, Inc.

C_{max} (2890.8-5915.7 ng/mL) and $AUC_{0\text{-}24h}$ (23,051.0-23,505.0 ng·h/mL) were ≥26- and 37-fold, respectively, higher than C_{max} and AUC_{last} achieved at the recommended clinical dose.

On the basis of the above results, the applicant considered it appropriate to set the maximum dose at 2000 mg/kg/day in the 13-week preliminary carcinogenicity study in rats.

(b) Thirteen-week preliminary carcinogenicity study in rats (4.2.3.4.1-5: Study 621[redacted]060)

The vehicle or acotiamide (1000, 1500, 2000 mg/kg/day) was administered orally to F344 rats (n = 10/sex/dose) for 13 weeks. There was no death caused by acotiamide. Animals in all acotiamide groups showed miosis and salivation. Males in the ≥1500 mg/kg/day dose groups showed decreases in erythrocytic parameters, decreases in total protein and albumin, while females in the same dose groups showed a decrease in the relative weight of the liver. Males in the 2000 mg/kg/day group showed decreases in triglycerides and in the relative weight of the liver, while females in the same dose group showed a decreased blood glucose level and an increase in heart weight, etc. Histopathological examination did not detect any effect of acotiamide. The TK study showed that, in the maximum dose group (2000 mg/kg/day), C_{max} (2846.8-4734.4 ng/mL) and $AUC_{0\text{-}24h}$ (16,096.1-31,338.5 ng·h/mL) were ≥26- and 25-fold, respectively, higher than C_{max} and AUC_{last} achieved at the recommended clinical dose.

On the basis of the above results, the applicant considered it appropriate to set the maximum dose at 2000 mg/kg/day in the carcinogenicity study in rats.

(c) Twenty-four-month carcinogenicity study in rats (4.2.3.4.1-6: Study 621[redacted]054)

The vehicle or acotiamide (200, 600, 2000 mg/kg/day) was administered orally to F344 rats (n = 50/sex/dose) for 24 months (study period from 20[redacted] to 20[redacted]). There was no death caused by acotiamide. Animals in the acotiamide groups showed miosis, salivation, lacrimation, and reddish tears. As a neoplastic change, endometrial adenocarcinoma was observed in 1 of 50 animals (2%) in the vehicle group, 5 of 50 animals (10%) in the 200 mg/kg/day group, 8 of 50 animals (16%) in the 600 mg/kg/day group, and 5 of 50 animals (10%) in the 2000 mg/kg/day group. The increase in the 600 mg/kg/day group was statistically significant. Although endometrial adenocarcinomas did not show clear dose dependence, in all acotiamide groups, the incidence of endometrial adenocarcinomas was higher than the laboratory's historical data obtained during the study period ([redacted]%-[redacted]%, 20[redacted] to 20[redacted]). As proliferative lesions in the uterus, cystic endometrial hyperplasia (6 of 50 animals in the vehicle group, 14 of 50 animals in the 200 mg/kg/day group, 10 of 50 animals in the 600 mg/kg/day group, 10 of 50 animals in the 2000 mg/kg/day group) and endometrial epithelial/glandular hyperplasia (4 of 50 animals in the vehicle group, 7 of 50 animals in the 200 mg/kg/day group, 4 of 50 animals in the 600 mg/kg/day group, 8 of 50 animals in the 2000 mg/kg/day group) were observed. The incidence of these lesions tended to increase in the acotiamide groups, but did not show clear dose-dependence.

On the basis of the above results, "a 2-step uterine carcinogenesis study in rasH2 mice" was conducted as described below in order to investigate the effect of acotiamide to modify the occurrence of endometrial adenocarcinomas.

3.(iii).A.(4).3) Two-step uterine carcinogenicity study in rasH2 mice

(a) Preliminary 2-step uterine carcinogenicity study in rasH2 mice (4.2.3.4.2-1: Study 657[redacted]004 [non-GLP])

ENU (120 mg/kg) was administered intraperitoneally in a single dose to female rasH2 wild-type mice[33] (n = 10 per group) and, at 1 week after administration of a single dose of ENU, the vehicle or acotiamide (600, 2000 mg/kg/day) was administered orally for 15 days. No death occurred in any of the groups. Acotiamide did not have any effect on the clinical observations, body weight, or findings of gross pathological examination.

From the above results, the applicant determined that the maximum dose of 2000 mg/kg/day used in this preliminary study would be tolerated in the main study with a treatment duration of up to 26 weeks.

(b) Two-step uterine carcinogenicity study in rasH2 mice (4.2.3.4.2-2: Study 657[redacted]005 [non-GLP])

ENU (120 mg/kg) was administered intraperitoneally in a single dose to female rasH2 mice (n = 50 per group) and, starting from after 1 week, the vehicle or acotiamide (2000 mg/kg/day) was administered orally for 26 weeks.[34] Animals died or were moribund-sacrificed from Week 12 in the ENU alone group and from Week 8 in the acotiamide group. At the end of the administration (27 weeks after ENU administration), the numbers of deaths and moribund-sacrifices were 19 of 50 animals (38%) and 24 of 50 animal (48%), respectively. There was no difference between the ENU alone group and the acotiamide group in clinical observations, body weight, food consumption, or organ weight. Macroscopic pathological examination of the uterus showed enlarged uterine horn (unilateral, bilateral), cyst (1 in either side), unilateral or bilateral discolored macules, discolored regions (1 or more), nodes/mass (unilateral, 1 or more), discolored nodes/mass (1 in either side, more than 1 in both sides), cervical cyst (1), and nodes/mass (1), but none of them showed statistically significant difference in frequency between the ENU alone group and the acotiamide group. Examination of other tissues and organs also showed various findings, nodes/mass in particular, but there was no statistically significant difference in the incidence between the ENU alone group and the acotiamide group.

The histopathological examination of the uterus showed the following changes (given in the order of ENU alone group and acotiamide group): atypical hyperplasia in 40 of 50 animals (80%) and in 43 of 50 animals (86%), cystic endometrial hyperplasia in 39 of 50 animals (78%) and in 40 of 50 animals (80%), adenocarcinoma in 5 of 50 animals (10%) and in 5 of 50 animals (10%), hemangioma in 17 of 50 animals (34%) and in 20 of 50 animals (40%), and angiosarcoma in 4 of 50 animals (8%) and in 5 of 50 animals (10%). The incidence of endometrial adenocarcinomas was the same (10%) in the two groups, and none of the findings showed statistically significant differences between the groups. Also, the mean number of atypical hyperplasias per animal was the same (1.8) between the two groups. Similarly, when data obtained from animals that died or were moribund sacrificed during the study and from those necropsied according to the protocol were tabulated separately, no statistically significant difference was observed between the two groups in the histopathological findings of the uterus or the mean number of atypical hyperplasia

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[33] Transgenic mice transduced with proto-oncogene of human origin (c-Ha-ras) (CByB6F1-Tg (HRAS) 2Jic)

[34] In order to identify the optimal timing for necropsy, a satellite group consisting of 36 female rasH2 mice was included in the study. Animals in this group received a single dose of ENU (120 mg/kg) intraperitoneally and, after 1 week, received repeated oral administration of the vehicle for 24 weeks. Histopathological examination of the uterus was performed on 4 to 5 animals each at 13, 15, 17, 19, 22, 23, and 24 weeks after ENU administration to monitor for the presence or absence of endometrial adenocarcinomas over time. As a result, no endometrial adenocarcinomas were observed up to Week 24 in the satellite group. In contrast, among animals that died or were moribund-sacrificed by Week 24 (19 animals in control group, 24 animals in acotiamide group), endometrial adenocarcinomas were observed in 1 animal each in the control group and in the acotiamide group. From these results, the timing of necropsy after acotiamide administration was set at 26 weeks, the standard test duration in carcinogenicity studies in transgenic mice.

per animal.

On the basis of the above results, the applicant determined that acotiamide does not affect ENU-induced uterine carcinogenesis in rasH2 mice.

3.(iii).A.(5) Reproductive and developmental toxicity

As reproductive and developmental toxicity studies, a study on fertility and early embryonic development to implantation in rats, embryo-fetal development studies in rats and rabbits, and a study on pre- and postnatal development, including maternal function in rats were conducted. In the study on embryo-fetal development in rabbits, decreased body weight and increased number of dwarfs were observed in fetuses at dose that showed decreased body weight due to decreased food consumption in maternal animals. Except these findings, there were no effects of acotiamide on the reproductive capacity of parent animals, embryo-fetal development, or growth and development of neonates. As was the case with the repeated-dose toxicity studies, miosis caused by the pharmacological action of acotiamide was observed in parent animals.

3.(iii).A.(5).1) Study on fertility and early embryonic development to implantation in rats (4.2.3.5.1-1: Study 603[redacted]051)

The vehicle or acotiamide (100, 300, 1000 mg/kg/day) was administered orally to male rats from 28 days prior to mating until the completion of mating (for 64-66 days) and to female rats from 14 days prior to mating until Gestation Day 7 (for 23-39 days). There was no death caused by acotiamide. Miosis was observed in both males and females at ≥300 mg/kg/day groups, and a decrease in the relative liver weight was observed in females of the 1000 mg/kg/day group. No organic changes were observed in the male or female reproductive organ in any of the treatment groups. Acotiamide had no effect on copulation rate, fertilization rate, estrous cycle of females, sperm analysis, number of luteal bodies, number of live embryos, or number of dead embryos. The NOAEL is determined to be 100 mg/kg/day for general toxicity in parent animals (male and female), 1000 mg/kg/day for reproductive capacity in both males and females, and 1000 mg/kg/day for embryo development.

3.(iii).A.(5).2) Study on embryo-fetal development in rats (4.2.3.5.2-2: Study 603[redacted]066)

The vehicle or acotiamide (100, 300, 1000 mg/kg/day) was administered orally to pregnant rats from Gestation Day 7 to Gestation Day 17. No death occurred in maternal animals. Miosis and decreases in absolute and relative liver weight were observed in the 1000 mg/kg/day group. No abnormality was observed in fetuses. The NOAEL is determined to be 300 mg/kg/day for general toxicity in maternal animals and 1000 mg/kg/day in fetuses.

3.(iii).A.(5).3) Study on embryo-fetal development in rabbits (4.2.3.5.2-4: Study 603[redacted]071)

The vehicle or acotiamide (100, 300, 1000 mg/kg/day) was administered orally to pregnant rabbits from Gestation Day 6 to 18. There was no death of maternal animals caused by acotiamide. Suppression of body weight increase was observed in the 300 mg/kg/day group, decreased food intake was observed in the ≥300 mg/kg/day dose groups, and decreased body weight, decreased ovary weight, abortion, and an increase in the number of maternal animals with premature labor were observed in the 1000 mg/kg/day group. In fetuses, decreased body weight and increased number of dwarfs were observed in the 1000 mg/kg/day group. The applicant considered that the effects observed in fetuses were changes secondary to the decreased body weight of maternal animals caused by decreased food intake. The NOAEL is determined to be 100 mg/kg/day for general toxicity and for maintaining pregnancy in maternal animals, and 300 mg/kg/day in fetuses.

3.(iii).A.(5).4) Study on pre- and postnatal development, including maternal function in rats (4.2.3.5.3-2: Study 621[redacted]055)

The vehicle or acotiamide (100, 300, 1000 mg/kg/day) was administered orally to pregnant rats from Gestation Day 7 to Lactation Day 21 (time of weaning). No death occurred in maternal animals (F_0). No changes were noted in clinical observations during the pregnancy or during the lactation. Acotiamide had no effect on pups (F_1) or fetuses (F_2). The open-field test on the function and behavior of pups (F_1) showed increases in the number of defecations and in the mean number of defecations in males of the 1000 mg/kg/day group and an increase in the number of sections entered in females of the same dose group. All were changes observed only in 1 out of 2 tests performed and not common to both sexes, from which the applicant considered that they are not causally related to acotiamide. The NOAEL is determined to be 1000 mg/kg/day for general toxicity and reproductive capacity of maternal animals and for pups.

3.(iii).A.(6) Other toxicity studies

3.(iii).A.(6).1) Antigenicity study (4.2.3.7-1: Study 603[redacted]069)

In the antigenicity study, active systemic anaphylaxis (ASA) reaction, passive cutaneous anaphylaxis (PCA) sensitization, and PCA reaction were evaluated.

Male guinea pigs (n = 10 for ASA reaction, n = 10 for PCA sensitization, n = 2 for PCA reaction) were sensitized by acotiamide (5 mg/body/day) by oral administration 15 times in 3 weeks or by subcutaneous administration 3 times in 3 weeks. At 2 weeks after the last sensitization, acotiamide (5 mg/body/day) was administered intravenously. As a result, no ASA reaction was induced. Serum samples were collected 29 days after the start of sensitization and subjected to test for the presence of antibody against acotiamide by PCA reaction. Results were negative.

Male BALB/c mice (n = 5 for PCA sensitization, n = 2 for PCA reaction) were sensitized by acotiamide by oral administration at 2 mg/body/day 15 times in 3 weeks or by intraperitoneal administration at 0.2 or 2 mg/body/day 3 times in 3 weeks. Serum samples were collected 29 days after the start of sensitization and subjected to a test for the presence of antibody against acotiamide by PCA reaction. Results were negative.

On the basis of the above results, the applicant determined that acotiamide is not antigenic in mice, rats, or guinea pigs under the experimental conditions used.

3.(iii).A.(6).2) Study on uterine enlargement (4.2.3.7.3-1: Study 656[redacted]010 [non-GLP])

In the 24-month carcinogenicity study in rats (4.2.3.4.1-6), endometrial adenocarcinomas were observed at a high frequency in the acotiamide group. In order to elucidate the mechanism of the occurrence, a study on uterine enlargement was conducted in which acotiamide was administered to 17 to 18 day-old female rats for 3 days, according to the method of using immature female rats stipulated in OECD Guideline 440 "Uterotrophic Bioassay in Rodents".[35]

The vehicle, acotiamide (200, 600, 1000 mg/kg), or a positive control ethinyl estradiol[36] (1.0 μg/kg) was administered once daily for 3 days to 17 to 18 day-old female rats (n = 6 per group). There was no change in food consumption. Animals in the acotiamide 1000 mg/kg group showed suppression of body weight increase. Animals in the positive control group showed increased uterine weight, thickening of the endometrial epithelium, mitotic figures,

[35] Juvenile animals have a complete hypothalamic-pituitary-gonadal axis (HPG axis). Therefore, the uterotrophic bioassay using juvenile rodents is considered to be an evaluation system highly responsive not only to substances that act on the uterus directly but also to those which affect HPG axis.

[36] Estrogen receptor agonist

occurrence of apoptosis, stratified and squamous vaginal mucosal epithelium, and increased percentage of PCNA[37]-positive cells in the endometrial epithelium. None of these findings were observed in any of the acotiamide groups. Thus, acotiamide did not affect the endometrial proliferation. On the basis of these results, the applicant considered that acotiamide is unlikely to have a direct growth-promoting effect on the uterus or to induce endocrine abnormality via the hypothalamic-pituitary-gonadal axis (HPG).

3.(iii).B Outline of the review by PMDA

3.(iii).B.(1) AChE inhibitory effect, the pharmacological action of acotiamide

The toxicity studies showed miosis which was caused by AChE inhibition, the pharmacological action of acotiamide. It is generally known that the AChE inhibitory effect is characterized by a decrease in brain cholinesterase levels observed at a dose far below that required for the manifestation of the usual toxicity. Therefore, PMDA asked the applicant whether or not brain cholinesterase was measured in the toxicity studies conducted, and if not, to state the reason for not conducting the measurement, and to explain the effect of acotiamide on the brain cholinesterase levels. PMDA also asked the applicant to explain the indices used for the reversibility of AChE inhibition in the toxicity studies and the appropriateness of the indices.

The applicant responded as follows:
Brain AChE activity was not measured in the toxicity studies on acotiamide. AChE inhibitors are roughly classified into irreversible inhibitors and reversible ones according to the mode of the inhibition. Irreversible AChE inhibitors, to which most organophosphorus insecticides belong, inactivate AChE by binding strongly with its active site. Therefore, the decreased AChE activity is not recovered until new AChE is synthesized (*Casarett and Doull's Toxicology: The Basic Science of Poisons,* 3rd ed.: 554-557). In contrast, the decreased AChE activity that is caused by reversible AChE inhibitors such as acotiamide is rapidly recovered when the inhibitor is removed from the reaction system with AChE (the site where AChE is present) (*Casarett and Doull's Toxicology: The Basic Science of Poisons*, 3rd ed.: 554-557). Therefore, the extent of AChE inhibition is considered to be dependent on the tissue concentration of the inhibitor.

When tissue radioactivity was measured in male rats following administration of ^{14}C-labeled acotiamide, the level of acotiamide-derived radioactivity was undetectable in the cerebrum and ≤1/55th the plasma concentration in the cerebellum. In addition, the clearance of initial uptake into the brain in male rats was comparable to, or less than, that of inulin [see "3.(ii).A.(2).1) Tissue distribution following single oral dose" and "3.(ii).A.(2).5).(d) Study on the clearance after initial uptake into gastric tissue"]. These findings suggest that acotiamide is distributed in the central nervous system to only a minimal extent. In addition, in the repeated-dose toxicity study in rats, the mean C_{max}[38] in males receiving the maximum dose (1000 mg/kg/day) was 2410 ng/mL. When the findings that acotiamide concentration in the cerebellum was ≤1/55th the plasma acotiamide concentration are extrapolated, the brain acotiamide concentration is calculated to 43.7 ng/mL (9.6×10^{-8} mol/L). This concentration is ≤1/24th the IC_{50} value (2.3×10^{-6} mol/L) against rat stomach-derived AChE [see "3.(i).A.(1).3).(a) AChE inhibitory activity and the mode of inhibition"]. In addition, by using the C_{max} value achieved at the recommended clinical dose of acotiamide and the brain-plasma concentration ratio (1/55th) described above, the brain acotiamide concentration is calculated to be 1.98 ng/mL (4.4×10^{-9} mol/L). This level is ≤1/130th and 1/610th, respectively, the inhibition constant measured using human recombinant AChE (6.1×10^{-7} mol/L in competitive inhibition, 2.7×10^{-6} mol/L in non-competitive inhibition) [see "3.(i).A.(1).3).(a) AChE inhibitory activity and the mode of inhibition"]. These results suggest that acotiamide administration is unlikely to decrease AChE activity in the brain.

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[37] Proliferating cell nuclear antigen

[38] The highest plasma concentration (C_{max} on Day 1 in the 4-week repeated-dose toxicity study) among the results obtained from TK analysis on male rats in repeated-dose toxicity studies (4 weeks, 6 months) conducted

Thus, although AChE activity in the brain was not measured in the toxicity studies conducted for the regulatory submission, acotiamide is extremely unlikely to inhibit AChE in the brain because (1) acotiamide inhibits AChE in a reversible manner and the intensity of the inhibition is considered to depend on the tissue concentration of acotiamide, and (2) acotiamide is distributed in the central nervous system only to a minimal extent and there will be little or no increase in acotiamide concentration in the brain. Furthermore, in the clinical studies, the incidence of adverse events related to nervous system disorders was comparable between the placebo group and the acotiamide group, and there were not any particular safety problems related to the nervous system in human subjects.

The reversibility of AChE inhibition was assessed by changes in clinical observations, such as miosis, lacrimation, and salivation, which are commonly observed when AChE is inhibited. These findings were marked in rats and dogs in the toxicity study of acotiamide. Therefore, the applicant considers that they are considered to be appropriate as indices for the reversibility.

PMDA accepts the explanation of the applicant. However, it is necessary to collect information on the occurrence of events caused by cholinergic actions via post-marketing surveillance, etc., and to carefully monitor and collect safety information from patients to whom acotiamide is concomitantly administered with cholinergic or anti-cholinergic drugs for which precautions for concomitant use is required [for the details of post-marketing surveillance, etc., see "4.(iii).B.(9) Post-marketing surveillance, etc."].

3.(iii).B.(2) Carcinogenicity of acotiamide

PMDA, by pointing out the finding that the incidence of endometrial adenocarcinomas was significantly higher in the acotiamide 600 mg/kg/day group compared with the control group in the 24-month carcinogenicity study in rats, asked the applicant to discuss and explain the relationship between acotiamide and endometrial adenocarcinomas based on the current scientific level.

The applicant explained as follows:
In rats of the same strain as that used in carcinogenicity studies, the incidence of endometrial adenocarcinomas tends to increase year by year. In the laboratory that conducted carcinogenicity studies of acotiamide in rats, the mean incidence of spontaneous endometrial adenocarcinomas was [redacted]% (range [redacted]%-[redacted]%, [redacted] studies) from 19[redacted] to 19[redacted] and [redacted]% (range [redacted]%-[redacted]%, [redacted] studies) from 20[redacted] to 20[redacted], showing a similar trend.

It is reported that drug-induced endometrial adenocarcinomas develop after a precancerous lesion caused by the direct exposure of the uterus to a carcinogenic substance or by a long-lasting endocrine abnormality such as hormone imbalance (*J Toxicol pathol.* 1999;12: 1-11). Relationship between acotiamide and endometrial adenocarcinomas was discussed from these standpoints.

In the carcinogenicity studies on acotiamide in rats, there was no clear difference in proliferative lesions of the mammary gland or endometrium, ovarian atrophy, or findings of vaginal epithelium between the control group and the acotiamide group, showing no findings suggestive of the involvement of estrogen-like effect in the acotiamide group. In the carcinogenicity studies in rats, although the plasma concentration of unchanged acotiamide in the acotiamide group tended to increase in a dose-dependent manner, the number of animals with endometrial adenocarcinomas did not increase with dose, neither was there any dose-dependent increase in

the number of findings suggestive of endocrine abnormality or precancerous lesions.[39]

Similarly, in the toxicity studies of acotiamide, except carcinogenicity studies, findings suggestive of genotoxicity, hormone imbalance, or proliferative lesions of the reproductive or related organs were not observed. From the aspect of the pharmacological action as well, in light of the findings that acotiamide is distributed in the central nervous system only to a minimal extent and that, although acotiamide may have a weak effect on dopamine receptor [see "3.(i).A.(1).3).(j) Effect on other receptors, etc."], oral administration of 1000 mg/kg to rats did not cause any change in plasma prolactin concentration.[40] From these results, acotiamide is unlikely to induce endometrial adenocarcinomas via hormones by excessive pharmacological action. In the pharmacokinetic studies as well, the concentration of acotiamide in the uterus after oral administration of acotiamide (10 mg/kg) to pregnant rats was similar to the plasma radioactivity concentration, ruling out the possibility of acotiamide specifically accumulating in the reproductive organ and exhibiting its effect [see "3.(ii).A.(2).2) Placental transfer in rats"].

Furthermore, in order to investigate the effect of acotiamide on hormones and on the possible proliferative effect on the endometrial membrane, an uterine enlargement study in rats, which is established as an *in vivo* system to assay estrogen activity, was conducted [see "3.(iii).A.(6).2) Study on uterine enlargement"]. None of the acotiamide groups (200, 600, 1000 mg/kg) showed changes in uterine weight or histopathological changes of the uterus as those observed in the positive control group treated with ethinyl estradiol. These results suggest that acotiamide is very unlikely to directly enhance the growth of the uterus or induce endocrine abnormality via HPG axis.

On the basis of the above results, the applicant considered that acotiamide is unlikely to be related to endometrial adenocarcinomas, and that the increased incidence of endometrial adenocarcinomas observed in the carcinogenicity studies in rats should be regarded as a spontaneous phenomenon. With the aim of further corroborating the above assumption, a 2-step carcinogenicity study was conducted in rasH2 mice to investigate whether or not acotiamide modifies the occurrence of endometrial adenocarcinomas. This is based on the report that, when a carcinogenic substance ENU (120 mg/kg) was administered intraperitoneally to rasH2 mice in a single dose, endometrial adenocarcinomas were induced in 16.7% (3 of 18 animals) after 22 weeks and in 55.6% (5 of 9 animals) after 24 weeks (*Cancer letters.* 2002;188:39-46, *J Toxicol Pathol*. 2002;15:145-151). Results of the study were as described in "3.(iii).A.(4).3) Two-step uterine carcinogenesis study in rasH2 mice". Thus, acotiamide did not have any modifying effect on the occurrence of endometrial adenocarcinomas.

On the basis of the above results, the applicant determined that endometrial adenocarcinomas observed in the acotiamide group in the 24-month carcinogenicity study in rats were spontaneous.

PMDA considers as follows:
Regarding the cause for the increase in the incidence of endometrial adenocarcinomas in the acotiamide group compared with the control group in the carcinogenicity study in rats, the explanation of the applicant at the regulatory submission was that since, in recent years, the incidence of endometrial adenocarcinomas in rats of the same strain tended to increase, the observed endometrial adenocarcinomas were spontaneous and there was no possibility of

[39] In the 24-month carcinogenicity study in rats (Study [redacted]) conducted in the laboratory that tested carcinogenicity of acotiamide from 19[redacted] to 20[redacted], the incidence of hyperplasia of uterine glandular epithelium was [redacted]% to [redacted]% for endometrial hyperplasia, [redacted]% to [redacted]% for hyperplasia of endometrial gland, [redacted]% to [redacted]% for cystic hyperplasia of endometrium, and [redacted]% to [redacted]% for adenoma.

[40] Male rats were given the vehicle (0.5%w/v MC solution) or acotiamide (10, 100, 1000 mg/kg) in a single oral dose, or left untreated, and plasma prolactin concentration was measured after 0.5, 1, 2, and 4 hours.

acotiamide being involved in the occurrence. PMDA considered it difficult to conclude that observed endometrial adenocarcinomas were spontaneous solely based on this finding. Instead, PMDA considered it necessary to closely examine the possibility of the involvement of acotiamide in the occurrence of endometrial adenocarcinomas, and has asked the applicant to thoroughly investigate the types, severity, frequency, etc., of pathological findings including non-neoplastic lesions observed in the female reproductive organ and endocrine-related organs in animals with uterine adenoma or adenocarcinoma and in animals without these neoplastic lesions in the carcinogenicity studies.

It is generally known that a majority of diseases in the reproductive system in rats are caused by changes in estrogen level. Also, changes in estrogen level easily activate prolactin cells in the pituitary gland, resulting in development of prolactin-secreting tumor after cell proliferation (*Casarett & Doull's Toxicology*. 6th ed. Scientist Press Co., Ltd.). Therefore, the applicant conducted the following additional investigations on the possibility of estrogen-like effect of acotiamide. In the 24-month carcinogenicity study in rats, close examination for non-neoplastic lesions in the endocrine organs and tissues such as pituitary gland, mammary gland, and uterus did not reveal any clear difference between the placebo group and the acotiamide group. Also, in none of the toxicity studies administering acotiamide, findings suggestive of changes in estrogen level or related proliferative lesions were observed. Furthermore, in the study on uterine enlargement in rats, which is commonly used as an *in vivo* study to detect estrogen-like effect of environmental hormones, acotiamide did not exhibit estrogen-like effect. On the basis of these results, the applicant considered that acotiamide is extremely unlikely to have estrogen-like activity.

On the other hand, regarding experimental uterine carcinogenesis models of mice and rats, it takes a long period of time to induce the cancer. Nontheless, it is known that the incidence is low (*J Toxicol Pathol*. 1999;12:1-11, *Toxicological Sciences* 2000;58:43-49, The Research Project Subsidized by the Health and Labour Sciences Research Grant for FY 1998, Partial Research Report Experimental studies on modifying effects of endocrine-disrupting chemicals in foods on carcinogenesis, *Toxicologic pathology* p. 244). Therefore, an additional study was conducted to investigate the activity of acotiamide to modify the occurrence of endometrial adenocarcinomas, using an experimental system that allows the development of endometrial adenocarcinomas by administering ENU to rasH2 mice, a system that induces the cancer at the highest frequency possible in a relatively short period of time at the current scientific level. As a result, administration of ENU alone induced endometrial adenocarcinomas in 10% of the animals. Acotiamide had no modifying effect whatsoever on the endometrial adenocarcinoma-inducing effect of ENU during the process of the cancer development, with respect to acceleration of the time of the occurrence, increase in the incidence, or the extent of malignancy of uterine adenocarcinoma that developed. Neither did acotiamide have any effect on proliferative lesions including hyperplasia.

It is known that uterine histiocytic sarcomas observed in the carcinogenicity study in mice occur spontaneously in some mouse strains such as B6C3F1, but it has not been reported in rats. Histogenesis of this tumor remains largely unknown, but it is also reported to be unrelated to hormones (*Histopathology of Toxicologic Pathology*, Japanese Society of Toxicologic Pathology, p. 329).

Based on the above discussion and the results of the additional study, PMDA concluded that there is little evidence to suspect that acotiamide increased the incidence of endometrial adenocarcinomas observed in the carcinogenicity study in rats, and that it is more appropriate to consider that the cancer occurs spontaneously, as observed usually in this rat strain.

4. Clinical data

4.(i) Summary of biopharmaceutic studies and associated analytical methods

4.(i).A Summary of the submitted data

Of the clinical study data submitted as the evaluation data in the application, those of Japanese clinical studies were obtained using 50 mg and 100 mg tablets. Eventually, 100 mg tablets were used as the proposed commercial formulation. In the foreign study on the effect on QT/QTc interval (TQT study), 300 mg tablets were used. In the mass balance study, a mixture of ^{14}C-labeled and unlabeled acotiamide hydrochloride hydrate (acotiamide) was used.

Plasma and fecal concentrations of unchanged acotiamide and the metabolite deisopropyl acotiamide were measured by high performance liquid chromatography-tandem mass spectrometry (LC/MS/MS), and urinary concentrations were measured by high performance liquid chromatography with UV detector (HPLC-UV). The lower limit of quantitation is shown as follows: 0.05 ng/mL for simultaneous measurement of plasma concentrations of unchanged acotiamide and deisopropyl acotiamide, and 1.00 ng/mL for measurement of plasma concentration of unchanged acotiamide alone; 10 ng/mL (standard method) and 50 ng/mL (enzyme treatment method[41]) for urinary concentration of unchanged acotiamide, and 20 ng/mL (standard method) and 50 ng/mL (enzyme treatment method[41]) for urinary concentration of deisopropyl acotiamide; and 0.04 μg/mL for fecal concentrations of both unchanged acotiamide and deisopropyl acotiamide. In the mass balance study, metabolites were analyzed by high performance liquid chromatography equipped with a radioisotope detector (HPLC-RI).

4.(i).A.(1) Dissolution test of drug products with different contents (5.3.1.2-2: Study 605[redacted]084 [[redacted] 20[redacted] to [redacted] 20[redacted]])

Acotiamide 50 mg and 100 mg tablets fall under the level [redacted] stipulated by the "Guideline for Bioequivalence Studies for Different Strengths of Oral Solid Dosage Forms (PFSB/ELD Notification No. 1124004 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, dated November 24, 2006). Therefore, a dissolution test was performed as required for the drug products of this level. Results confirmed the equivalence of the dissolution profiles between the drug products.

4.(i).A.(2) Food effect study (5.3.3.1-5: Study [redacted] [[redacted] 20[redacted] to [redacted] 20[redacted]])

A 6-group, 3-dosage regimen, 3-period cross-over study was conducted at a single center in Japan involving healthy adult male subjects aged ≥20 and <45 years (target sample size of 30) to evaluate the food effect on the pharmacokinetics of acotiamide and safety of acotiamide.

Acotiamide (100 mg) was to be administered under fasting conditions, before a meal, or after a meal, and a washout period of ≥5 days was included between the treatment periods.

All of 30 treated subjects were included in the pharmacokinetic[42] and safety analysis sets.

Regarding safety, adverse events were observed in 40.0% (12 of 30 subjects) (16.7% [5 of 30 subjects] after fasted administration, 17.2% [5 of 29 subjects] after preprandial administration, 17.2% [5 of 29 subjects] after postprandial administration). The only adverse event observed in ≥2 subjects in any administration condition was "blood triglycerides increased" (2 subjects after fasted administration, 3 subjects after preprandial administration, 3 subjects after postprandial administration). The only adverse event for which causal relationship with the study drug was not ruled out (adverse drug reaction) was "enteritis infectious and white blood cell count

[41] Enzymatic treatment with β-glucuronidase

[42] One subject discontinued the study after fasted administration because of an adverse event. Therefore, summary statistics of pharmacokinetic parameters were calculated in 29 subjects.

increased" in 1 subject after postprandial administration. There were no deaths or serious adverse events.

Table 20 shows the pharmacokinetic parameters of unchanged acotiamide in plasma. Table 21 shows the geometric mean ratio (90% confidence interval [CI]) of C_{max} and AUC_{last} (preprandial vs. fasted administration, postprandial vs. fasted administration, and postprandial vs. preprandial administration).

Table 20. Plasma pharmacokinetic parameters of unchanged acotiamide

	C_{max} (ng/mL)	AUC_{last} (ng·h/mL)	t_{max} (h)	$t_{1/2}$ (h)
Fasted administration	42.20 ± 18.08	143.51 ± 49.69	1.20 ± 0.92	16.9 ± 9.6
Preprandial administration	69.24 ± 30.56	133.36 ± 30.67	0.96 ± 0.34	36.5 ± 21.2
Postprandial administration	41.37 ± 20.57	107.99 ± 31.29	1.67 ± 0.72	34.2 ± 8.9

Mean ± SD, n = 29

Table 21. Evaluation of food effect

	C_{max} (ng/mL)	AUC_{last} (ng·h/mL)
Preprandial/fasted	1.627 [1.380, 1.918]	0.959 [0.879, 1.047]
Postprandial/fasted	0.970 [0.823, 1.144]	0.768 [0.703, 0.838]
Postprandial/preprandial	0.596 [0.506, 0.703]	0.800 [0.733, 0.874]

Geometric mean ratio [90% CI], n = 29

4.(i).B Outline of the review by PMDA

4.(i).B.(1) Food effect

The applicant explained the results of the food effect study as follows:

In the food effect study in healthy adult male subjects conducted in Japan, C_{max} was the highest after preprandial administration compared with fasting or postprandial administration, with the level being higher by 62.7% compared with that after fasted administration. AUC_{last} was the lowest after postprandial administration, being 76.8% and 80.0%, respectively, of the level after fasted administration and preprandial administration. As shown in the results of food effect given in Table 21, 90% CIs of the geometric mean ratio of C_{max} and AUC_{last} were outside the range from 0.8 to 1.25, except the ratio of C_{max} after postprandial administration to that after fasted administration and the ratio of AUC_{last} after preprandial administration to that after fasted administration.

On the basis of the above results, the applicant determined that the pharmacokinetics of unchanged acotiamide is affected by food consumption.

PMDA considers that, in the phase II and phase III studies conducted in patients with FD, acotiamide was administered before a meal, the administration method which resulted in the highest level of C_{max} in the food effect study, and accordingly preprandial administration is proposed in the application. Therefore, there should be no major concerns about overexposure due to noncompliance of the timing of food consumption.

Since the pharmacokinetics of unchanged acotiamide after treatment is affected by the conditions of food consumption, the information on the results of the food effect study should be appropriately provided via the package insert.

4.(ii) Summary of clinical pharmacology studies

4.(ii).A Summary of the submitted data

4.(ii).A.(1) *In vitro* studies with human-derived samples

Results of *in vitro* studies with human-derived samples are described under "3.(ii).A.(1) Absorption," "3.(ii).A.(2) Distribution," "3.(ii).A.(3) Metabolism," and "3.(ii).A.(5) Pharmacokinetic drug-drug interactions."

4.(ii).A.(2) Japanese phase I single-dose study

(5.3.3.1-1: Study [redacted] [redacted] to [redacted])

A placebo-controlled, double-blind study was conducted at a single center in Japan to evaluate the safety and pharmacokinetics of a single oral dose of acotiamide in healthy adult male subjects aged ≥20 and <55 years (target sample size of 18).

Placebo or acotiamide (50, 100, 200, 400, 800 mg) was to be administered to subjects in group A (placebo, acotiamide 50 mg, 200 mg, 800 mg) and group B (placebo, acotiamide 100 mg, 400 mg) orally under fasting conditions in the alternating order of A→B→A→B→A, with dose escalation from a lower dose.[43] Subjects were to proceed to the next step of treatment after an interval of at least 10 days.

All of 19 treated subjects (10 subjects in group A, 9 subjects in group B) were included in the safety and pharmacokinetic analysis sets.

No adverse events were observed.

Table 22 shows pharmacokinetic parameters of unchanged acotiamide and deisopropyl acotiamide in plasma. The urinary excretion rate up to 48 hours after administration was 0.02331% to 0.07356% for unchanged acotiamide and 0.000% to 0.002383% for deisopropyl acotiamide. The fecal excretion rate up to 72 hours after administration was 1.925% to 81.22%[44] for unchanged acotiamide and 0.009060% to 2.511%[44] for deisopropyl acotiamide.

Table 22. Plasma pharmacokinetic parameters of unchanged acotiamide and deisopropyl acotiamide

	Dose (mg)	C_{max} (ng/mL)	AUC_{last} (ng·h/mL)	CL/F (L/h)	t_{max} (h)	$t_{1/2}$ (h)
Unchanged acotiamide	50	16.37 ± 13.49	49.42 ± 9.988	823.1 ± 167.9	2.75 ± 2.59	12.90 ± 4.96
	100	30.82 ± 13.33	161.7 ± 54.11	537.0 ± 181.5	2.42 ± 0.97	13.31 ± 6.91
	200	72.40 ± 34.44	306.9 ± 101.5	580.8 ± 184.6	2.08 ± 1.24	10.92 ± 4.44
	400	185.9 ± 116.9	683.3 ± 141.1	464.1 ± 119.2	2.25 ± 1.21	21.69 ± 12.18
	800	318.4 ± 122.9	1480 ± 379.0	440.3 ± 136.8	2.13 ± 0.89	17.14 ± 13.16
Deisopropyl acotiamide	50	0.1523 ± 0.05014	0.4134 ± 0.1611[a)]		2.75 ± 2.59	4.33 ± 3.40[a)]
	100	0.2533 ± 0.09212	1.001 ± 0.3957		2.67 ± 1.25	3.22 ± 1.69
	200	0.7322 ± 0.4818	2.424 ± .1.051		1.92 ± 1.24	4.94 ± 3.73
	400	2.416 ± 1.262	10.19 ± 1.443		1.58 ± 0.86	7.38 ± 3.24
	800	3.713 ± 1.039	18.79 ± 6.143		1.75 ± 0.92	7.71 ± 2.06

Mean ± SD, n = 6

a) n = 5

[43] Subjects in group A were to receive 3 doses (1 dose of placebo and 2 doses of acotiamide). Subjects in group B were to receive 2 doses (2 doses of acotiamide, or 1 dose each of placebo and acotiamide).

[44] The applicant considers that the excretion rate varied substantially from subject to subject due to the difference in the amount of the feces recovered among the subjects.

Urine samples were treated with β-glucuronidase and the urinary excretion rate was measured before and after the enzyme treatment. As a result, the rate increased after enzyme treatment both for unchanged acotiamide and for deisopropyl acotiamide. The urinary excretion rate was very low both for unchanged acotiamide and for deisopropyl acotiamide. Therefore, the applicant considered that both unchanged acotiamide and deisopropyl acotiamide in urine are present mostly in the form of glucuronide conjugates.

4.(ii).A.(3) Japanese phase I multiple-dose study
(5.3.3.1-2: Study [redacted] [redacted] to [redacted])

A placebo-controlled, double-blind study was conducted at a single center in Japan to evaluate the safety and pharmacokinetics of multiple oral administration of acotiamide in healthy adult male subjects aged ≥20 and <55 years (target sample size of 18).

Placebo or acotiamide (100, 300 mg) was to be administered three times daily (daily dose 300 mg and 900 mg, respectively) before a meal. The subjects received a single dose of the study drug in the morning on Day 1, followed by 1-day washout period (Day 2), and then received the study drug three times daily from Day 3 for 7 days. On the last administration day (Day 9), the subjects received the study drug only in the morning.

All of 18 treated subjects were included in the safety and pharmacokinetic analysis sets.

Regarding safety, adverse events were observed in 16.7% (1 of 6 subjects) ("ALT increased") in the placebo group, in 16.7% (1 of 6 subjects) ("ALT increased and blood prolactin increased") in the 100 mg/dose group, and in 33.3% (2 of 6 subjects) ("ALT increased" and "blood prolactin increased" in 1 subject each) in the 300 mg/dose group. The causal relationship of the study drug to these adverse events was not ruled out. There were no deaths or serious adverse events.

Table 23 shows the pharmacokinetic parameters of unchanged acotiamide and deisopropyl acotiamide in plasma. The urinary excretion rates up to 48 hours after the last dose in the 100 mg/dose and 300 mg/dose groups were 0.05648% and 0.09798%, respectively, for unchanged acotiamide; and 0.000% and 0.002084%, respectively, for deisopropyl acotiamide.

Table 23. Plasma pharmacokinetic parameters of unchanged acotiamide and deisopropyl acotiamide

	Dose (mg/dose)	Day	C_{max} (ng/mL)	AUC_{last} (ng·h/mL)	CL/F (L/h)	t_{max} (h)	$t_{1/2}$ (h)
Unchanged acotiamide	100	1	56.69 ± 44.03	125.3 ± 36.60	698.6 ± 179.5	1.00 ± 0.00	10.45 ± 3.91
		9	45.94 ± 20.07	207.3 ± 43.50	417.4 ± 102.9	1.54 ± 1.72	8.03 ± 1.64
	300	1	200.0 ± 140.4	537.2 ± 236.0	515.3 ± 175.4	1.17 ± 0.26	8.99 ± 3.80
		9	168.2 ± 66.40	1215 ± 398.9	222.7 ± 74.97	1.58 ± 0.20	11.25 ± 2.74
Deisopropyl acotiamide	100	1	0.3151 ± 0.2491	0.5542 ± 0.2729		1.08 ± 0.20	2.78 ± 0.63
		9	0.3033 ± 0.1549	1.152 ± 0.5259		1.00 ± 0.32	4.25 ± 2.73
	300	1	1.204 ± 0.8316	2.670 ± 1.382		1.25 ± 0.27	3.98 ± 1.57
		9	1.322 ± 0.6090	9.321 ± 3.502		1.50 ± 0.00	12.63 ± 8.651

Mean ± SD, n = 6

Comparison of the ratio of 6-β-hydroxycortisol and cortisol excreted in urine on Day 1 and Day 9 showed no significant difference between Day 1 and Day 9 in any of the groups. On the basis of the above results, the applicant considered that acotiamide has little effect on CYP3A4.

4.(ii).A.(4) Long-term treatment studies (5.3.5.2-1, 5.3.5.2-2: Study [redacted] [redacted] to [redacted])

For the outline of the studies, see "4.(iii).A.(2).2) Long-term treatment study."

Plasma concentration of unchanged acotiamide was measured at Week 4, 12, 24, 36, and 48, or at study discontinuation. A total of 347 subjects with measurable plasma acotiamide concentration at 1 or more time points were included in the pharmacokinetic analysis set, and among them, 283 subjects who met the criteria for the timing of pharmacokinetic analysis[45] were included in the analysis of drug exposure level.

Drug exposure level was analyzed using a linear mixed effects model as the base model with the timing of pharmacokinetic analysis and time after the last dose as fixed effects and subject as the random effect. During the course of constructing the final model, sex, age, and body weight were evaluated as candidates for covariates affecting the pharmacokinetics of acotiamide. None of them had a significant effect. Therefore, using the above model as the final model, the geometric mean ratios [95% CI] for the plasma acotiamide concentrations at Weeks 12 and 36 to the concentration at Week 4 were calculated. As a result, the ratios were 1.151 [0.993, 1.334] for Week 12 vs. Week 4 and 1.149 [0.912, 1.447] for Week 36 vs. Week 4, which suggested that the drug exposure level remained constant throughout the treatment period.

An exploratory population pharmacokinetic (PPK) analysis was conducted in 319 subjects[46] to search for covariates that significantly affected the pharmacokinetic parameters of acotiamide (factors examined: age, body weight, sex, ALT, AST, serum albumin, total bilirubin, creatinine clearance, use or non-use of concomitant drugs). None of these factors significantly affected CL/F.

4.(ii).A.(5) Study on gastric emptying (5.3.4.1-1: Study [redacted] [redacted] to [redacted])

A placebo-controlled, 2-group, 2-period, cross-over study was conducted at a single center in Japan to evaluate the gastric emptying after a single oral dose of acotiamide in healthy adult male subjects aged ≥20 and ≤29 years (target sample size of 12).

Placebo or acotiamide (100 mg), or placebo or acotiamide (300 mg), was to be administered in a single oral dose before a meal. A washout period of 6 days was included between the treatment periods.

All of 12 treated subjects were included in the safety and efficacy (gastric emptying) analysis sets.

Regarding safety, the only adverse event observed was "blood triglycerides increased" in 1 subject in the placebo group; the causal relationship with the study drug was not ruled out. There were no deaths or serious adverse events.

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[45] Drug concentration data obtained from blood samples collected within the period from 1.5 to 12 hours after the last dose were analyzed. Drug concentration data below the lower limit of quantitation and data obtained from subjects for whom a steady state of pharmacokinetics could not be assumed because of non-compliance or unknown compliance were excluded from analysis.

[46] Subjects who took the study drug and had at least one blood sample collected for pharmacokinetic evaluation, from whom those with problems with GCP compliance and those with unknown treatment compliance were excluded. Drug concentrations below the lower limit of quantitation limit and data obtained from subjects with non-compliance or unknown compliance were excluded.

Gastric emptying was evaluated by breath test.[47] Table 24 shows the parameters of gastric emptying calculated from gastric emptying speed (% dose/h)[48] over time. Although some of the subjects showed changes in gastric emptying parameters after acotiamide administration, there was no significant difference between the placebo group and the acotiamide 100 mg or 300 mg group in the data of gastric emptying parameters.

Table 24. Parameters related to gastric emptying

Dose group	Study drug	$t_{1/2ex}$ (min)	$t_{lag\ ex}$ (min)	GEC
100 mg	Placebo	131.00 ± 24.71	78.67 ± 19.61	3.313 ± 0.206
	100 mg	133.28 ± 22.46	80.82 ± 15.22	3.280 ± 0.252
300 mg	Placebo	129.15 ± 20.86	79.13 ± 14.23	3.385 ± 0.242
	300 mg	126.70 ± 22.41	74.50 ± 12.17	3.315 ± 0.238

Mean ± SD, n = 6
$t_{1/2ex}$: time to 50% excretion of the total cumulative ^{13}C excreted into exhaled air
$t_{lag\ ex}$: time to reach the peak of the % dose/h curve
GEC: gastric emptying coefficient which reflects the percentage of the appearance and disappearance of ^{13}C in exhaled air

4.(ii).A.(6) Ultrasonography study (5.3.4.2-1: Study [redacted] [redacted])

A randomized, placebo-controlled, double-blind, parallel group, comparative study was conducted at a single center in Japan to evaluate the effect of acotiamide on the motility of the stomach and the duodenum in patients (aged ≥20 and ≤79 years) diagnosed with FD[49] in accordance with the Rome II criteria[50] (target sample size of 40).

After the run-in period of 8 to 14 days, placebo or acotiamide (300 mg) was to be orally administered daily in 3 divided doses before a meal for 14 to 18 days of the treatment period.

All of 42 treated subjects (21 subjects in the placebo group, 21 subjects in 300 mg/day group) were included in the safety analysis set and in the full analysis set (FAS) for the primary efficacy analysis.

The effect on the motility of the stomach and the duodenum was evaluated using, as indices, the cross-sectional area of the proximal stomach (stomach's ability to expand), gastric emptying rate (gastric emptying), motility index (activity to contract the gastric antrum), and reflux coefficient (duodenogastric reflux) measured by external ultrasonography (Table 25). Tables 26 and 27 show results. No significant difference was observed between the placebo group and the acotiamide group in any of these parameters.

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47 At 30 minutes after the study drug administration, the test meal containing ^{13}C-labeled sodium acetate was given to each subject, and exhaled air was collected continuously for 4 hours.

48 Percentage of ^{13}C exhaled into air relative to the total dose of ^{13}C administered.

49 Patients who repeatedly showed any one of 7 symptoms (upper abdominal pain, upper abdominal discomfort, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting) from 6 months or more before informed consent. They had to show at least 2 symptoms out of the above 7 symptoms, in a persistent or recurrent manner, for at least 2 weeks during 2 months before informed consent. They also had to show at least 2 moderate or severe symptoms (these should include one or more of postprandial fullness, upper abdominal bloating, and early satiation) that occurred on not less than 2 days (twice) from 7 days to 1 day before the day of receiving ultrasonography.
Patients with chief complaint of heartburn, patients with irritable bowel syndrome, and patients who had moderate or severe heartburn during the run-in period were excluded.

50 See Table 31.

Table 25. Outline of the procedure for ultrasonography

Cross-sectional area of the proximal stomach	Determined by placing an ultrasound caliper between the ribs under the left armpit on the abdomen of the subject lying in supine position in such a way as to visualize the spleen.
Gastric emptying rate	Calculated by applying the value for the cross-sectional area of the gastric antrum (Determined by placing an ultrasound caliper between the ribs under the left armpit on the abdomen of the subject lying in supine position in such a way as to visualize the spleen) to the following equation: Gastric emptying rate (%) = ("cross-sectional area of the relaxed gastric antrum at 6 minutes after taking 400 mL of the test meal*" – "cross-sectional area of the relaxed gastric antrum at 15 minutes after taking 400 mL of the test meal") / ("cross-sectional area of the relaxed gastric antrum at 6 minutes after taking 400 mL of the test meal") × 100 *: Consomme soup
Motility index	Gastric contraction rate was calculated by applying the value for the cross-sectional area of the gastric antrum at each time point to the following equation: Contraction rate = ("cross-sectional area of the gastric antrum at maximum relaxation at 6 to 9 minutes after taking 400 mL of the test meal" – "cross-sectional area of the gastric antrum at maximum contraction at 6 to 9 minutes after taking 400 mL of the test meal") / ("cross-sectional area of the gastric antrum at maximum relaxation at 6 to 9 minutes after taking 400 mL of the test meal") Motility index was calculated by applying, to the following equation, the value for the frequency of gastric antrum contraction observed for 3 minutes from 6 to 9 minutes after taking 400 mL of the test meal. Motility index = contraction rate × frequency
Reflux coefficient	Calculated by applying, to the following equation, the frequency of duodenogastric reflux observed for 5 minutes from 9 to 14 minutes after taking 400 mL of the test meal) and the observed mean reach of the reflux signal from the pyloric ring: Reflux coefficient = frequency × mean reach of reflux signal from the pyloric ring
Procedure	
Method for taking the study drug and the test meal: Before the start of the treatment period, the patient took approximately 100 mL of water in sitting position at 30 minutes before the scheduled timing of starting to take the test meal and, at 30 minutes after taking water, took 100 mL of the test meal 4 times every 3 minutes in supine position. After the end of the treatment period (or at study discontinuation), the patient took the study drug together with approximately 100 mL of water in sitting position at 30 minutes before the scheduled timing of starting to take the test meal and, at 30 minutes after taking the study drug, took 100 mL of the test meal 4 times every 3 minutes in supine position. 1. The baseline area before taking the test meal and the areas at steady state after taking 0, 100, 200, 300, and 400 mL of the test meal in a cumulative manner were measured. 2. Cross-sectional area of the relaxed gastric antrum at 6 minutes after taking 400 mL of the test meal was measured. The cross-sectional area of the gastric antrum at the maximum relaxation and at the maximum contraction, and the number of gastric antrum contractions, during 3 minutes from 6 minutes after taking 400 mL of the test meal, were measured at the cross section of the gastric antrum. 3. An ultrasound caliper was placed on the abdomen in such a way as to show the cross section that visualizes the gastric antrum, pyloric ring, and duodenal bulb simultaneously, and the mean reach of the reflux signal from the pyloric ring, and the number of duodenogastric reflux during 5 minutes from 9 minutes after taking 400 mL of the test meal were measured. 4. An ultrasound caliper was placed on the abdomen in such a way as to visualize the abdominal aorta and the superior mesenteric artery, and the cross-sectional area of the relaxed gastric antrum at 15 minutes after taking 400 mL of the test meal was measured.	

Table 26. Ultrasonographic evaluation (Cross-sectional area of the proximal stomach [cm^2])

Amount of test meal intake		0 mL	100 mL	200 mL	300 mL	400 mL
Placebo group (N = 19)	Before intake	4.430 ± 3.357	14.408 ± 5.954	23.305 ± 7.641	32.112 ± 11.900	38.297 ± 13.977
	After intake	3.898 ± 2.600	13.205 ± 5.124	22.088 ± 5.269	29.563 ± 7.712	37.764 ± 10.274
	Change	-0.532 ± 3.589	-1.203 ± 6.493	-1.216 ± 7.311	-2.549 ± 8.950	-0.533 ± 10.503
300 mg/day group (N = 19)	Before intake	3.230 ± 2.774	15.968 ± 6.937	24.328 ± 7.823	30.553 ± 10.108	35.703 ± 12.383
	After intake	4.601 ± 4.182	15.432 ± 5.962	23.566 ± 7.331	31.377 ± 7.426	38.970 ± 9.400
	Change	1.371 ± 4.387	-0.537 ± 7.764	-0.763 ± 9.905	0.824 ± 11.016	3.267 ± 13.859

Mean ± SD
Two subjects in each group were handled as those with missing data because of noncompliance with the timing for the test, etc., precluding the comparison of test data between before and after intake.

Table 27. Ultrasonographic evaluation (gastric emptying rate, motility index, reflux coefficient)

		Gastric emptying rate (%)	Motility index	Reflux coefficient
		Placebo group: 19 subjects 300 mg/day group: 19 subjects	Placebo group: 19 subjects 300 mg/day group: 18 subjects	Placebo group: 18 subjects 300 mg/day group: 18 subjects
Placebo group	Before intake	52.32 ± 24.32	8.85 ± 0.73	16.5 ± 12.9
	After intake	57.74 ± 23.73	8.78 ± 1.31	18.7 ± 15.2
	Change	5.42 ± 21.33	-0.07 ± 1.46	2.2 ± 18.7
300 mg/day group	Before intake	50.91 ± 21.10	8.92 ± 0.99	25.6 ± 12.0
	After intake	65.43 ± 21.94	8.99 ± 0.48	29.3 ± 21.1
	Change	14.52 ± 20.55	0.07 ± 0.84	3.7 ± 17.1

Mean ± SD
Because of noncompliance with the timing for the test, etc., precluding the comparison of test data between before and after test meal intake, the following number of subjects were handled as those with missing data: 2 subjects in each group for gastric emptying rate, 2 subjects in the placebo group and 3 subjects in the 300 mg/day group for motility index, and 3 subjects in each group for reflux coefficient.

Regarding safety, adverse events were observed in 33.3% (7 of 21 subjects) in the placebo group and in 28.6% (6 of 21 subjects) in the 300 mg/day group, and adverse drug reactions were observed in 14.3% (3 of 21 subjects) in the placebo group and in 19.0% (4 of 21 patients) in the 300 mg/day group. Adverse events observed in ≥2 subjects in any group were "blood prolactin increased" (4.8% [1 of 21 subjects] in the placebo group, 9.5% [2 of 21 subjects] in the 300 mg/day group) and "blood triglycerides increased" (0.0% [0 of 21 patients] in the placebo group, 9.5% [2 of 21 subjects] in the 300 mg/day group). The only adverse drug reaction observed in ≥2 subjects in any group was "blood prolactin increased" (4.8% [1 of 21 subjects] in the placebo group, 9.5% [2 of 21 subjects] in the 300 mg/day group). There were no deaths or serious adverse events.

4.(ii).A.(7) Foreign mass balance study (5.3.3.1-3: Study [redacted] [redacted])

An open label study was conducted at a single center in a foreign country to investigate the mass balance, identify metabolites, and evaluate the safety, following a single oral dose of ^{14}C-labeled acotiamide in healthy adult male subjects aged ≥18 and ≤45 years (target sample size of 6).

A mixture of ^{14}C-labeled and unlabeled acotiamide (600 mg) was to be administered in a single

oral dose.

All of 6 treated subjects were included in the pharmacokinetic and safety analysis sets.

Regarding safety, adverse events were observed in 66.7% (4 of 6 subjects) ("headache" in 2 subjects, "loose stools, haematochezia, and haemorrhoids" in 1 subject, "loose stools" in 1 subject). Adverse drug reactions were observed in 66.7% (4 of 6 subjects) ("loose stools" and "headache" in 2 subjects each). There were no deaths or serious adverse events.

Regarding pharmacokinetics, radioactivity was detected in the blood and in the plasma at 0.25 hour after administration of ^{14}C-labeled acotiamide, the first blood sampling point after administration. Mean AUC_{last} of radioactivity concentration and unchanged acotiamide in the plasma was 2122.1 ng Eq·h/mL and 1271.4 ng·h/mL, respectively, with unchanged acotiamide accounting for approximately 60% of the radioactivity in plasma. The urinary and fecal excretion rates of radioactivity up to 216 hours after ^{14}C-labeled acotiamide administration were approximately 5.3% and 92.7%, respectively, of total radioactivity administered.

4.(ii).A.(8) Foreign clinical study on the effect on QT/QTc interval
(5.3.3.1-4: Study [redacted] [redacted] 20[redacted] to [redacted] 20[redacted])

A randomized, double-blind (open-label for positive control only), 4-group, 4-period, cross-over study was conducted at a single center in a foreign country to investigate the effect of multiple oral administration of acotiamide on QTcF interval in healthy adult male and female subjects aged ≥18 and ≤50 years (target sample size of 80).

Subjects were assigned to 4 treatment groups (A-D) as shown in Table 28. A washout period of ≥5 days was included between the treatment periods.

Table 28. Dosage regimen

Treatment group	Dosage regimen	
A	Day 1-4	Placebo 3 times daily before each meal
	Day 5	Placebo once daily before breakfast
B	Day 1-4	Acotiamide 300 mg 3 time daily before each meal (900 mg/day)
	Day 5	Acotiamide 300 mg once daily before breakfast
C	Day 1-4	Acotiamide 900 mg 3 time daily before each meal (2700 mg/day)
	Day 5	Acotiamide 900 mg once daily before breakfast
D	Day 1-4	Placebo 3 times daily before each meal
	Day 5	Moxifloxacin 400 mg once daily before breakfast

Of 80 treated subjects, 8 subjects discontinued the study (consent withdrawal [2 subjects], adverse events [2 subjects], protocol deviations [4 subjects]). Depending on the timing of study discontinuation, 79 subjects were included in the pharmacokinetic analysis set,[51] and 73 subjects in the electrocardiographic analysis set.[52]

Regarding the change in QTcF interval from baseline (least squares mean), the upper limit of the one-sided 95% CI of the difference between the placebo group and the 300 or 900 mg/dose group was <10 msec at all measuring time points, which demonstrated that acotiamide did not prolong QTc interval. In contrast, comparison between the placebo group and the moxifloxacin showed that the lower limit of the one-sided 95% CI exceeded 5 msec at 2 and 4 hours after

[51] The population of subjects from whom a sufficient amount of samples for pharmacokinetic parameter calculation was collected, as determined by the person responsible for pharmacokinetic analysis

[52] Subjects who, in addition to placebo administration, completed administration of acotiamide or moxifloxacin for at least 1 period

administration, confirming that the test system has sufficient assay sensitivity.

Table 29 shows the pharmacokinetic parameters on Day 5 of acotiamide administration.

Table 29. Pharmacokinetic parameters of acotiamide

	No. of subjects	C_{max} (ng/mL)	AUC_{tau} (ng·h/mL)	t_{max} (h)
300 mg/dose group	74	156.47 ± 64.131	740.14 ± 266.609	3.19 ± 1.571
900 mg/dose group	76	451.59 ± 215.666	1659.57 ± 494.480	3.05 ± 1.360

Mean ± SD

4.(ii).B Outline of the review by PMDA

4.(ii).B.(1) Drug-drug interactions

Results of the *in vitro* studies suggest that CYP2C8, CYP1A1, CYP3A4, UGT1A8, and UGT1A9 are mainly involved in the metabolism of acotiamide [see "3.(ii).A.(3).6) Study on CYP isoforms involved in metabolism" and "3.(ii).A.(3).7) Study on UGT isoforms involved in metabolism"]. PMDA asked the applicant to explain the possibility that the concomitant use of acotiamide with inhibitors of these enzymes would affect the pharmacokinetics of acotiamide.

The applicant responded as follows:
In the study conducted using human UGT-expressing microsomes, acotiamide was metabolized to M-1 (glucuronide conjugate of acotiamide) by UGT1A8 and UGT1A9 [see "3.(ii).A.(3).7) Study on UGT isoforms involved in metabolism"]. There are only scanty reports on the involvement of UGT enzymes in drug-drug interactions in clinical practice. Since acotiamide is metabolized by the two UGT isoforms, the metabolism to M-1 will not be inhibited completely even if either of the enzymes were to be inhibited by a concomitant drug. Therefore, plasma acotiamide concentration is unlikely to increase to a level that poses safety concerns.

On the other hand, in a separate *in vitro* metabolic study, acotiamide was metabolized to M-4 (deisopropyl acotiamide) in the highest yield by CYP2C8, followed by CYP1A1, and then by CYP3A4 [see "3.(ii).A.(3).6) Study on CYP isoforms involved in metabolism"]. However, CYP content in human liver varies depending on isoform; CYP2C8 is present at a concentration of only 64 pmol CYP/mg, whereas CYP3A4 is expressed at a high concentration (108 pmol CYP/mg), and CYP1A1 is expressed only at a very low level unless induced (*Biochem Pharmacol.* 1998;56:377-387, *Biochem Pharmacol.* 1999;57:465-480). Thus, it is suggested that CYP2C8 and CYP3A4 are mainly involved in the metabolism of acotiamide to M-4. However, results of the mass balance study showed that the combined urinary and fecal excretion rate of M-2 and M-4 was only 4.98% of the acotiamide dose administered. In addition, the two metabolic enzymes appear to be mainly involved in the metabolism. These findings suggest that pharmacokinetics of acotiamide will not be significantly affected even if either one of the enzymes is completely inhibited.

Additionally, PPK analysis in the long-term treatment study ([redacted]) showed that "use or non-use of concomitant drugs", which was investigated as a candidate for covariate of interindividual variability in pharmacokinetic parameters, did not significantly affect CL/F. Furthermore, in the late phase II study ([redacted]), the phase III study ([redacted]), and the long-term treatment study ([redacted]), no clinically relevant adverse events were observed in patients to whom acotiamide was administered in combination with drugs that are known to serve as substrates or inhibitors for CYP3A4 or UGT1A9.

On the basis of the above results, the applicant considered that pharmacokinetics of acotiamide is unlikely to be affected significantly by the concomitant use of acotiamide with drugs that

serve as substrates or inhibitors for key metabolic enzymes of acotiamide.

PMDA considers that the explanation of the applicant is acceptable. However, the information on the key metabolic enzymes of acotiamide needs to be appropriately provided via the package insert.

4.(iii) Summary of clinical efficacy and safety

4.(iii).A Summary of the submitted data

As the main efficacy and safety evaluation data, the results from a total of 5 studies consisting of 3 Japanese phase II studies, 1 Japanese phase III study, and 1 long-term treatment study, were submitted (Table 30). Each study was conducted in patients with FD that met the definition of Table 31. Efficacy was evaluated according to the criteria shown in Table 32.

Table 30. Summary of clinical studies submitted

Type of study	Study design (treatment duration)	Dose	Subjects	Results of primary endpoints or primary variables
Phase IIa study	Placebo-controlled, randomized, double-blind, comparative study (4 weeks)	Placebo, Acotiamide 150 mg/day, Acotiamide 300 mg/day, Acotiamide 900 mg/day	Patients diagnosed with FD in accordance with the Rome II criteria	Subject's general impression at the last evaluation time point (percentage of patients who felt better) (FAS) Placebo group: 64.5% (20/31) 150 mg/day group: 75.0% (24/32) 300 mg/day group: 84.8% (28/33) 900 mg/day group: 76.7% (23/30)
Phase IIb1 study	Placebo-controlled, randomized, double-blind, comparative study (4 weeks)	Placebo, Acotiamide 300 mg/day, Acotiamide 900 mg/day	Patients diagnosed with FD in accordance with the Rome II criteria	Improvement rate of subject's general impression at the last evaluation time point (FAS) Placebo group: 41.7% (43/103) 300 mg/day group: 51.5% (52/101) 900 mg/day group: 49.5% (50/101)
Phase IIb2 study	Placebo-controlled, randomized, double-blind, comparative study (4 weeks)	Placebo, Acotiamide 150 mg/day, Acotiamide 300 mg/day, Acotiamide 900 mg/day	Patients diagnosed with FD in accordance with the Rome II criteria	Improvement rate of subject's general impression at the last evaluation time point (FAS) Placebo group: 49.1% (55/112) 150 mg/day group: 48.7% (56/115) 300 mg/day group: 58.3% (63/108) 900 mg/day group: 56.9% (66/116)
Phase III study	Placebo-controlled, randomized, double-blind, comparative study (4 weeks)	Placebo, Acotiamide 300 mg/day	Patients diagnosed with FD in accordance with the Rome III criteria	Improvement rate of subject's general impression at the last evaluation time point in the treatment period (FAS) Placebo group: 34.8% (154/442) 300 mg/day group: 52.2% (235/450) Elimination rate of 3 symptoms at the last evaluation time point in the treatment period (FAS) Placebo group: 9.0% (40/442) 300 mg/day group: 15.3% (69/450)
Long-term treatment study	Open-label, uncontrolled study (24 weeks up to 48 weeks)	Acotiamide 300 mg/day	Patients diagnosed with FD in accordance with the Rome III criteria	Improvement rate of subject's general impression at Week 24 in the treatment period (FAS) 300 mg/day group: 48.9% (69/141) Elimination rate of 3 symptoms at the last evaluation time point (Week 8) in the treatment period (FAS) 300 mg/day group: 10.9% (44/405)

Table 31. Summary of criteria for FD

	Rome II	Rome III	Patients studied[a)]
Disease duration	Patients with the following symptoms for at least 12 weeks, which need not be consecutive, in the preceding 12 months.	The following criteria fulfilled for the last 3 months with symptom onset at least 6 months prior to diagnosis	Patients with the following symptoms from 6 or more months before informed consent
Symptoms	Persistent or recurrent gastrointestinal symptoms (pain or discomfort on the midline of the upper abdomen), excluding irritable bowel syndrome	At least 1 of the following symptoms: - Bothersome postprandial fullness - Early satiation, - Epigastric pain - Epigastric burning	At least 1 of the following symptoms: - Postprandial fullness - Early satiation - Upper abdominal pain - Upper abdominal discomfort
Organic disease	No evidence of organic disease (including at upper endoscopy) that is likely to explain the symptoms	No evidence of structural disease (including at upper gastrointestinal endoscopy) that is likely to explain the symptoms	Patients with confirmed organic disease (confirmed by upper gastrointestinal endoscopy) that is likely to explain the subjective symptom(s) were excluded.
Classification	- Ulcer-like - Dysmotility-like - Unspecified	- Epigastric pain syndrome - Postprandial distress syndrome	In Phase III study, patients with chief complaint of postprandial distress syndrome among Rome III criteria for FD were studied.
Main inclusion criteria in Phase III and long-term treatment studies			- Two or more of the following 8 symptoms were noted repeatedly from not less than 3 months before informed consent: upper abdominal pain, upper abdominal discomfort, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting, excessive belching. Either or both of postprandial fullness or early satiation had to be included in the observed symptoms. - Chief complaint confirmed at informed consent was postprandial fullness, upper abdominal bloating, or early satiation, among the above 8 symptoms. - During the 7-day run-in period, moderate or severe postprandial fullness, upper abdominal bloating, or early satiation occurred on at least 2 days (twice) (symptoms were evaluated using a 4-grade rating scale of none, mild, moderate, and severe). - The chief complaint confirmed on the day of upper gastrointestinal endoscopy (or at enrollment in the long-term treatment study) was postprandial fullness, upper abdominal bloating, or early satiation.
Main exclusion criteria in Phase III and long-term treatment studies			Patients who had heartburn within 12 weeks before informed consent Patients with concurrent irritable bowel syndrome Patients who had heartburn during the run-in period

a) For Phase IIa, Phase IIb1, and Phase IIb2 studies, see the outline of each study.

Table 32. Criteria for efficacy endpoints

Endpoint	Study	Evaluation method
Subject's general impression	Phase IIa study	To the question "How do you feel about your stomach conditions during the past 1 week compared with those during the run-in period (from informed consent to 1 day before upper gastrointestinal endoscopy)?", each subject rated his/her impression using a 3-grade rating scale (feeling better, unchanged, feeling worse) and recorded the result in the patient diary every week (or at study discontinuation). The percentage of subjects "feeling better" in the efficacy-evaluable population was calculated.
	Phase IIb1 study Phase IIb2 study Phase III study Long-term treatment study	To the question "how do you feel about your stomach conditions during the past 1 week compared with those during the run-in period (from informed consent to 1 day before upper gastrointestinal endoscopy)?", each subject rated his/her impression using a 7-grade rating scale (feeling much better, feeling better, feeling slightly better, unchanged, feeling slightly worse, feeling worse, feeling much worse) and recorded the result in the patient diary every week (or at study discontinuation). "Feeling much better" and "feeling better" were defined as "improved," and the percentage of subjects with "improved" symptoms in the efficacy-evaluable population as "improvement rate."
Elimination of 3 symptoms	Phase III study Long-term treatment study	Elimination of all 3 symptoms, postprandial fullness, upper abdominal bloating, and early satiation, was defined as "elimination", and the percentage of subjects with "elimination" in the efficacy-evaluable population as "elimination rate of 3 symptoms."

4.(iii).A.(1) Phase II studies

4.(iii).A.(1).1) Early phase II study (5.3.5.1-1: Study [redacted] [Phase IIa study] [redacted])

A multi-center, randomized, placebo-controlled, double-blind, parallel-group, comparative study was conducted at 20 centers in Japan to evaluate the efficacy and safety of acotiamide in patients (aged ≥20 and ≤79 years) diagnosed with FD in accordance with the Rome II criteria[53] (target sample size of 120).

After a run-in period of 8 to 14 days, placebo or acotiamide (150, 300, or 900 mg) was to be administered orally daily in 3 divided doses before a meal for 28 days of the treatment period.

All of 127 subjects receiving the study drug (32 subjects in the placebo group, 32 subjects in the 150 mg/day group, 33 subjects in the 300 mg/day group, 30 subjects in the 900 mg/day group) were included in the safety analysis set. Of these, 126 subjects (31 subjects in the placebo group, 32 subjects in the 150 mg/day group, 33 subjects in the 300 mg/day group, 30 subjects in the 900 mg/day group), excluding 1 subject in the placebo group who took the study drug for <7 days, were included in the FAS for the primary efficacy analysis.

Regarding efficacy, Table 33 shows the subject's general impression[54] and physician's evaluation[55] at the last evaluation time point.[56]

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[53] Patients who had any one of 8 symptoms (upper abdominal pain, upper abdominal discomfort, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting, excessive belching) within 2 months before giving informed consent and who had at least 2 moderate or severe symptoms during the run-in period. Patients with irritable bowel syndrome and patients who had moderate or severe heartburn during the run-in period were excluded.

[54] Each subject rated his/her general impression on the condition of his/her stomach using a 3-grade rating scale (feeling better, unchanged, feeling worse during the past 1 week compared with the condition during the run-in period) in the patient diary every week (or at study discontinuation). The value indicates the percentage of subjects who "felt better" among efficacy-evaluable subjects.

[55] The physician evaluated the efficacy using a 3-grade rating scale (improved, unchanged, worsened) at Week 4 or at subject's visit after study discontinuation, based on the collective judgment of the results entered in the patient diary and the results of the interview.

[56] At Week 4 or at study discontinuation

Table 33. Subject's general impression and physician's evaluation at the last evaluation time point (FAS)

Subject's general impression	Placebo group (N = 31)	150 mg/day group (N = 32)	300 mg/day group (N = 33)	900 mg/day group (N = 30)
Feeling better	64.5% (20)	75.0% (24)	84.8% (28)	76.7% (23)
Unchanged	32.3% (10)	21.9% (7)	15.2% (5)	23.3% (7)
Feeling worse	3.2% (1)	3.1% (1)	0.0% (0)	0.0% (0)
Physician's evaluation	Placebo group [a)] (N = 30)	150 mg/day group [a)] (N = 31)	300 mg/day group (N = 33)	900 mg/day group (N = 30)
Improved	70.0% (21)	77.4% (24)	93.9% (31)	76.7% (23)
Unchanged	30.0% (9)	22.6% (7)	6.1% (2)	23.3% (7)
Worsened	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)

a) Subjects handled as those with missing data because of violation of concomitant medication were excluded.

Regarding safety, adverse events were observed in 68.8% (22 of 32 subjects) in the placebo group, 56.3% (18 of 32 subjects) in the 150 mg/day group, 60.6% (20 of 33 subjects) in the 300 mg/day group, and 56.7% (17 of 30 subjects) in the 900 mg/day group. Adverse drug reactions were observed in 21.9% (7 of 32 subjects) in the placebo group, 18.8% (6 of 32 subjects) in the 150 mg/day group, 15.2% (5 of 33 subjects) in the 300 mg/day group, and 26.7% (8 of 30 subjects) in the 900 mg/day group. Tables 34 and 35 show adverse events and adverse drug reactions, respectively, which occurred in ≥2 subjects in any group. There were no deaths. "Asthma" was observed as a serious adverse event in 1 subject in the 300 mg/day group, but its causal relationship with the study drug was ruled out.

Table 34. Adverse events observed in ≥2 subjects in any group

	Placebo group (N = 32)		150 mg/day group (N = 32)		300 mg/day group (N = 33)		900 mg/day group (N = 30)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Overall	68.8%	22	56.3%	18	60.6%	20	56.7%	17
Blood triglycerides increased	18.8%	6	9.4%	3	9.1%	3	10.0%	3
Nasopharyngitis	3.1%	1	6.3%	2	9.1%	3	0.0%	0
Diarrhoea	0.0%	0	3.1%	1	9.1%	3	3.3%	1
Blood prolactin increased	9.4%	3	6.3%	2	3.0%	1	13.3%	4
Upper respiratory tract inflammation	6.3%	2	6.3%	2	3.0%	1	3.3%	1
γ-GTP increased	6.3%	2	6.3%	2	3.0%	1	0.0%	0
White blood cell count increased	6.3%	2	6.3%	2	3.0%	1	0.0%	0
Blood potassium decreased	3.1%	1	6.3%	2	0.0%	0	0.0%	0
Blood prolactin decreased	3.1%	1	6.3%	2	0.0%	0	0.0%	0
Dizziness	3.1%	1	0.0%	0	0.0%	0	6.7%	2
White blood cell count decreased	0.0%	0	0.0%	0	0.0%	0	6.7%	2
ALT increased	6.3%	2	0.0%	0	0.0%	0	3.3%	1

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Table 35. Adverse drug reactions observed in ≥2 subjects in any group

	Placebo group (N = 32)		150 mg/day group (N = 32)		300 mg/day group (N = 33)		900 mg/day group (N = 30)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Overall	21.9%	7	18.8%	6	15.2%	5	26.7%	8
Blood prolactin increased	6.3%	2	6.3%	2	3.0%	1	10.0%	3
Blood prolactin decreased	0.0%	0	6.3%	2	0.0%	0	0.0%	0
ALT increased	6.3%	2	0.0%	0	0.0%	0	3.3%	1

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4.(iii).A.(1).2) Late phase II study 1 (5.3.5.1-2: Study [redacted] [Phase IIb1 study] [[redacted] 20[redacted] to [redacted] 20[redacted]])

A multi-center, randomized, placebo-controlled, double-blind, parallel group, comparative study was conducted at 33 centers in Japan to evaluate the efficacy and safety of acotiamide in patients (aged ≥20 and ≤79 years) diagnosed with FD in accordance with the Rome II criteria[57] (target sample size of 300).

After a run-in period of 8 days, placebo or acotiamide (300 or 900 mg) was to be administered orally in 3 divided doses before a meal for 28 days of the treatment period.

Of 322 subjects receiving the study drug (107 subjects in the placebo group, 104 subjects in the 300 mg/day group, 111 subjects in the 900 mg/day group), 321 subjects (107 subjects in the placebo group, 104 subjects in the 300 mg/day group, 110 subjects in the 900 mg/day group), excluding 1 subject[58] in the 900 mg/day group who was judged by the investigator to be difficult to continue study participation, were included in the safety analysis set. Of 322 subjects receiving the study drug, 317 subjects (107 subjects in the placebo group, 104 subjects in the 300 mg/day group, 106 subjects in the 900 mg/day group), excluding 5 subjects[59] in the 900 mg/day group, were included in the FAS for the primary efficacy analysis.

Table 36 shows the results of the primary endpoint "improvement rate[60] of subject's general impression at the last evaluation time point.[56]" Cochran-Armitage test was performed using contrasts (-2, 1, 1), (-1, 1, 0), and (-1, 0, 1) for the placebo group, 300 mg/day group, and 900 mg/day group. Results showed no statistically significant difference (adjusted *P* values for contrasts [-2, 1, 1], [-1, 1, 0], and [-1, 0, 1] were 0.272, 0.324, and 0.474, respectively, at a two-sided level of significance 5%; adjusted for multiplicity of test by sample extraction method).

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57 Patients who had any one of 8 symptoms (upper abdominal pain, upper abdominal discomfort, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting, excessive belching) within 2 months before giving informed consent and who had at least 2 moderate or severe symptoms during the run-in period.
Patients with chief complaint of heartburn, patients with irritable bowel syndrome, and patients who had moderate or severe heartburn during the run-in period were excluded.

58 Subjects who never made a return visit during the treatment period. Although it was confirmed by phone that the subject took the study drug, the detail of the treatment compliance was unknown because the patient diary was lost and no safety information was available.

59 Three subjects who took the study drug for <7 days, 1 subject with unknown treatment compliance, and 1 subject who violated the exclusion criteria (took prohibited concomitant medication from before informed consent until study completion)

60 Subjects who felt "much better" or "better" compared with the condition during the run-in period were classified as those with "improved" conditions. The percentage of "improved" subjects in the efficacy-evaluable subjects was defined as the "improvement rate".

Table 36. Subject's general impression at the last evaluation time point (FAS)

Subject's general impression	Placebo group (N = 107)	300 mg/day group (N = 104)	900 mg/day group (N = 106)
No. of subjects analyzed [a)]	103	101	101
Feeling much better	12.6% (13)	16.8% (17)	11.9% (12)
Feeling better	29.1% (30)	34.7% (35)	37.6% (38)
Feeling slightly better	35.9% (37)	32.7% (33)	33.7% (34)
Unchanged	19.4% (20)	12.9% (13)	14.9% (15)
Feeling slightly worse	1.9% (2)	3.0% (3)	2.0% (2)
Feeling worse	1.0% (1)	0.0% (0)	0.0% (0)
Feeling much worse	0.0% (0)	0.0% (0)	0.0% (0)
Improvement rate [95% CI]	41.7% [32.6%, 51.3%]	51.5% [41.8%, 60.9%]	49.5% [39.9%, 59.0%]
Adjusted *P* value [b, c)]	(-2, 1, 1): *P* = 0.272, (-1, 1, 0): *P* = 0.324, (-1, 0, 1): *P* = 0.474		

a) Patients who took prohibited concomitant medications or did not make self-evaluation (4 subjects in the placebo group, 3 subjects in the 300 mg/day group, 5 subjects in the 900 mg/day group) were handled as those with missing data.

b) Cochran-Armitage test using contrasts (-2, 1, 1), (-1, 1, 0), and (-1, 0, 1) for the placebo group, 300 mg/day group, and 900 mg/day group; two-sided level of significance of 5%

c) *P* value adjusted for multiplicity of test by sample extraction method

Adverse events were observed in 50.5% (54 of 107 subjects) in the placebo group, 59.6% (62 of 104 subjects) in the 300 mg/day group, and 48.2% (53 of 110 subjects) in the 900 mg/day group. Adverse drug reactions were observed in 22.4% (24 of 107 subjects) in the placebo group, 26.9% (28 of 104 subjects) in the 300 mg/day group, and 20.0% (22 of 110 subjects) in the 900 mg/day group. Tables 37 and 38 show adverse events and adverse drug reactions, respectively, which were observed in ≥2.0% of subjects in any group. There were no deaths or serious adverse events.

Table 37. Adverse events observed in ≥2.0% of subjects in any group

	Placebo group (N = 107)		300 mg/day group (N = 104)		900 mg/day group (N = 110)	
	Incidence	N	Incidence	N	Incidence	N
Overall	50.5%	54	59.6%	62	48.2%	53
Blood triglycerides increased	10.3%	11	12.5%	13	7.3%	8
Blood prolactin increased	4.7%	5	12.5%	13	5.5%	6
Diarrhoea	4.7%	5	8.7%	9	5.5%	6
Headache	4.7%	5	7.7%	8	2.7%	3
Blood bilirubin increased	3.7%	4	5.8%	6	3.6%	4
ALT increased	3.7%	4	5.8%	6	1.8%	2
γ-GTP increased	2.8%	3	4.8%	5	3.6%	4
Blood potassium decreased	3.7%	4	3.8%	4	2.7%	3
Somnolence	0.9%	1	2.9%	3	1.8%	2
White blood cell count increased	0.0%	0	2.9%	3	0.9%	1
Nasopharyngitis	2.8%	3	1.9%	2	4.5%	5
Eczema	0.0%	0	1.9%	2	2.7%	3
Blood uric acid increased	0.9%	1	1.0%	1	2.7%	3
Nausea	2.8%	3	1.9%	2	0.0%	0
Dysmenorrhoea	4.7%	5	1.0%	1	0.0%	0

MedDRA/J ver.8.1

Table 38. Adverse drug reactions observed in ≥2.0% of subjects in any group

	Placebo group (N = 107)		300 mg/day group (N = 104)		900 mg/day group (N = 110)	
	Incidence	N	Incidence	N	Incidence	N
Overall	22.4%	24	26.9%	28	20.0%	22
Blood prolactin increased	3.7%	4	10.6%	11	3.6%	4
Diarrhoea	1.9%	2	2.9%	3	2.7%	3
ALT increased	0.9%	1	2.9%	3	1.8%	2

MedDRA/J ver.8.1

4.(iii).A.(1).3) Late phase II study 2 (5.3.5.1-3: Study [redacted] [Phase IIb2 study] [[redacted] 20[redacted] to [redacted] 20[redacted]])

A multi-center, randomized, placebo-controlled, double-blind, parallel group, comparative study was conducted at 46 centers in Japan to evaluate the efficacy and safety of acotiamide in patients (aged ≥20 and ≤79 years) diagnosed with FD in accordance with the Rome II criteria[61] (target sample size of 440).

After a run-in period of 8 days, placebo or acotiamide (150, 300, or 900 mg) was to be administered orally in 3 divided doses before a meal for 28 days of the treatment period.

Of 461 subjects receiving the study drug (115 subjects in the placebo group, 117 subjects in the 150 mg/day group, 111 subjects in the 300 mg/day group, 118 subjects in the 900 mg/day group), 454 subjects (112 subjects in the placebo group, 116 subjects in the 150 mg/day group, 109 subjects in the 300 mg/day group, 117 subjects in the 900 mg/day group) excluding 7 subjects[62] were included in the safety analysis set. Of 461 subjects receiving the study drug, 451 subjects (112 subjects in the placebo group, 115 subjects in the 150 mg/day group, 108 subjects in the 300 mg/day group, 116 subjects in the 900 mg/day group) excluding 10 subjects[63] were included in the FAS for the primary efficacy analysis.

Table 39 shows the results of the primary efficacy endpoint, "improvement rate of subject's general impression[60] at the last evaluation time point.[56]" Cochran-Armitage test was performed using contrasts (-3, -1, 1, 3) and (-5, -1, 3, 3) for the placebo group, 150 mg/day group, 300 mg/day group, and 900 mg/day group. Results did not show any statistically significant difference (adjusted one-sided *P* values for contrasts [-5, -1, 3, 3] and [-3, -1, 1, 3] were 0.075 and 0.069, respectively; one-sided level of significance of 2.5%, adjusted for multiplicity of test by sample extraction method).

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61 Patients who repeatedly showed any one of 7 symptoms (upper abdominal pain, upper abdominal discomfort, postprandial fullness, upper abdominal bloating, early satiation, nausea, vomiting) from 6 months or more before giving informed consent, and who had at least 2 of the above symptoms for at least 2 weeks during 2 months before giving informed consent. Subjects also had to have at least 2 moderate or severe symptoms (should include at least 1 of postprandial fullness, upper abdominal bloating, and early satiation) among the above 7 symptoms on at least 2 days (twice) within 7 days from informed consent to 1 day before upper endoscopy.
Patients who had heartburn within 2 months before informed consent, patients with irritable bowel syndrome, and patients who had heartburn during the run-in period were excluded.

62 Six subjects who were found to have been enrolled in duplicate and for whom data were judged unreliable at the case conference, and 1 subject who never made a return visit during the treatment period

63 Six subjects with double enrollment, 3 subjects who took the study drug for <7 days, 1 subject who met the exclusion criteria (found to have an organic disease)

Table 39. Subject's general impression at the last evaluation time point (FAS)

Subject's general impression	Placebo group (N = 112)	150 mg/day group (N = 115)	300 mg/day group (N = 108)	900 mg/day group (N = 116)
Feeling much better	15.2% (17)	10.4% (12)	17.6% (19)	21.6% (25)
Feeling better	33.9% (38)	38.3% (44)	40.7% (44)	35.3% (41)
Feeling slightly better	38.4% (43)	33.0% (38)	28.7% (31)	30.2% (35)
Unchanged	12.5% (14)	14.8% (17)	12.0% (13)	12.1% (14)
Feeling slightly worse	0.0% (0)	2.6% (3)	0.9% (1)	0.9% (1)
Feeling worse	0.0% (0)	0.9% (1)	0.0% (0)	0.0% (0)
Feeling much worse	0.0% (0)	0.0% (0)	0.0% (0)	0.0% (0)
Improvement rate [95% CI]	49.1% [40.0%, 58.2%]	48.7% [39.7%, 57.7%]	58.3% [48.9%, 67.1%]	56.9% [47.8%, 65.5%]
Adjusted one-sided *P* value [a, b)]	(-3, -1, 1, 3): *P* = 0.075, (-5, -1, 3, 3): *P* = 0.069			

a) Cochran-Armitage test using contrasts (-3, -1, 1, 3) and (-5, -1, 3, 3) for the placebo group, 150 mg/day group, 300 mg/day group, and 900 mg/day group; one-sided level of significance of 2.5%
b) *P* value adjusted for multiplicity of test by sample extraction method

Adverse events were observed in 50.9% (57 of 112 subjects) in the placebo group, 57.8% (67 of 116 subjects) in the 150 mg/day group, 55.0% (60 of 109 subjects) in the 300 mg/day group, and 50.4% (59 of 117 subjects) in the 900 mg/day group. Adverse drug reactions were observed in 15.2% (17 of 112 subjects) in the placebo group, 21.6% (25 of 116 subjects) in the 150 mg/day group, 21.1% (23 of 109 subjects) in the 300 mg/day group, and 17.9% (21 of 117 subjects) in the 900 mg/day group. Tables 40 and 41 show adverse events and adverse drug reactions, respectively, which were observed in ≥2.0% of subjects in any group, respectively. There were no deaths or serious adverse events.

Table 40. Adverse events observed in ≥2.0% of subjects in any group

	Placebo group (N = 112)		150 mg/day group (N = 116)		300 mg/day group (N = 109)		900 mg/day group (N = 117)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Overall	50.9%	57	57.8%	67	55.0%	60	50.4%	59
Nasopharyngitis	3.6%	4	6.0%	7	9.2%	10	6.0%	7
Blood triglycerides increased	11.6%	13	13.8%	16	8.3%	9	13.7%	16
Nausea	7.1%	8	2.6%	3	7.3%	8	1.7%	2
Blood bilirubin increased	0.9%	1	1.7%	2	6.4%	7	3.4%	4
Vomiting	3.6%	4	3.4%	4	5.5%	6	2.6%	3
Blood prolactin increased	1.8%	2	3.4%	4	5.5%	6	2.6%	3
Dyspepsia	5.4%	6	4.3%	5	4.6%	5	1.7%	2
Diarrhoea	6.3%	7	2.6%	3	4.6%	5	2.6%	3
Constipation	0.9%	1	3.4%	4	2.8%	3	0.0%	0
ALT increased	0.0%	0	3.4%	4	2.8%	3	0.0%	0
γ-GTP increased	3.6%	4	0.9%	1	2.8%	3	3.4%	4
Eructation	2.7%	3	2.6%	3	1.8%	2	1.7%	2
Platelet count increased	0.0%	0	2.6%	3	1.8%	2	0.0%	0
Dysmenorrhoea	2.7%	3	4.3%	5	0.9%	1	3.4%	4
Headache	3.6%	4	2.6%	3	0.9%	1	2.6%	3
Abdominal pain upper	3.6%	4	1.7%	2	0.9%	1	5.1%	6
White blood cell count increased	0.9%	1	0.9%	1	0.9%	1	2.6%	3
Pharyngolaryngeal pain	0.0%	0	1.7%	2	2.8%	3	0.9%	1
Abdominal discomfort	2.7%	3	0.9%	1	0.9%	1	0.9%	1
Pharyngitis	3.6%	4	1.7%	2	0.0%	0	0.0%	0

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Table 41. Adverse drug reactions observed in ≥2.0% of subjects in any group

	Placebo group (N = 112)		150 mg/day group (N = 116)		300 mg/day group (N = 109)		900 mg/day group (N = 117)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Overall	15.2%	17	21.6%	25	21.1%	23	17.9%	21
Blood prolactin increased	0.9%	1	3.4%	4	4.6%	5	2.6%	3
Diarrhoea	0.9%	1	0.9%	1	4.6%	5	0.9%	1
Constipation	0.9%	1	2.6%	3	1.8%	2	0.0%	0
γ-GTP increased	2.7%	3	0.0%	0	0.9%	1	2.6%	3
Blood triglycerides increased	3.6%	4	2.6%	3	0.0%	0	4.3%	5

MedDRA/J ver.10.0

4.(iii).A.(2) Phase III studies

4.(iii).A.(2).1) Phase III study (5.3.5.1-4: Study [redacted] [Phase III study] [redacted])

A multi-center, randomized, placebo-controlled, double-blind, parallel-group, comparative study was conducted at 67 centers in Japan to evaluate the efficacy and safety of acotiamide in patients (aged ≥20 and ≤64 years) diagnosed with FD in accordance with the Rome III criteria[64] (target sample size of 820).

After a run-in period of 8 days, placebo or acotiamide (300 mg) was to be administered orally daily in 3 divided doses before a meal for 28 days of the treatment period. Subjects were to undergo a 28-day post-treatment observation period without treatment.

All of 892 treated subjects (442 subjects in the placebo group, 450 subjects in the 300 mg/day group) were included in the safety analysis set and in the FAS for the primary efficacy analysis.

The primary efficacy endpoints were "improvement rate[60] of subject's general impression at the last evaluation time point in the treatment period[65]" and "elimination rate of 3 symptoms at the last evaluation time point in the treatment period.[66]" If a statistically significant difference was observed between the 300 mg/day group and the placebo group in both primary endpoints, the 300 mg/day group was to be determined superior to the placebo group. Table 42 shows the results. A statistically significant difference was observed between the 300 mg/day group and the placebo group in both primary endpoints ($P < 0.001$ and $P = 0.004$, respectively, Fisher's exact test, two-sided level of significance of 5%).

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64 See Table 31.

65 The last time point with data in the treatment period

66 When all 3 subjective symptoms, postprandial fullness, upper abdominal bloating, and early satiation, eliminated at the last evaluation time point in the treatment period, the subject was considered to have achieved elimination of symptoms, and the percentage of subjects with "elimination" to the efficacy-evaluable subjects was defined as "the elimination rate of 3 symptoms."

Table 42. Efficacy at the last evaluation time point in treatment period (FAS)

	Placebo group (N = 442)	300 mg/day group (N = 450)
Subjects with improvement	154	235
Improvement rate of subject's general impression [95% CI]	34.8% [30.5%, 39.3%]	52.2% [47.6%, 56.7%]
Between-group difference of improvement rate [95% CI]	–	17.4% [11.0%, 23.7%]
P value[a)]	–	$P < 0.001$
Subjects with elimination of 3 symptoms	40	69
Elimination rate of 3 symptoms [95% CI]	9.0% [6.7%, 12.0%]	15.3% [12.2%, 18.9%]
Between-group difference for elimination rate of 3 symptoms [95% CI]	–	6.3% [2.1%, 10.5%]
P value[a)]	–	$P = 0.004$

a) Fisher's exact test, two-sided level of significance of 5%

Regarding safety, adverse events were observed in 60.4% (267 of 442 subjects) in the placebo group and 56.0% (252 of 450 subjects) in the 300 mg/day group. Adverse drug reactions were observed in 18.1% (80 of 442 subjects) in the placebo group and 16.9% (76 of 450 subjects) in the 300 mg/day group. Table 43 shows adverse events that were observed in ≥2.0% of subjects in any group. The only adverse drug reactions observed in ≥2.0% of subjects in any group were "blood prolactin increased" (4.8% [21 of 442 subjects] in the placebo group, 3.6% [16 of 450 subjects] in the 300 mg/day group) and "diarrhoea" (2.3% [10 of 442 subjects] in the placebo group and 1.6% [7 of 450 subjects] in the 300 mg/day group). There were no deaths. The only serious adverse event observed was "intervertebral disc disorder" in 1 subject of the 300 mg/day group, and its causal relationship with the study drug was ruled out.

Table 43. Adverse events observed in ≥2.0% of subjects in any group

	Placebo group (N = 442)		300 mg/day group (N = 450)	
	Incidence	N	Incidence	N
Overall	60.4%	267	56.0%	252
Blood triglycerides increased	20.6%	91	18.9%	85
Nasopharyngitis	9.3%	41	8.7%	39
γ-GTP increased	6.3%	28	7.1%	32
Blood prolactin increased	6.8%	30	4.7%	21
Diarrhoea	4.1%	18	4.7%	21
Blood bilirubin increased	4.1%	18	4.2%	19
ALT increased	3.8%	17	4.0%	18
Dyspepsia	3.6%	16	3.8%	17
White blood cell count increased	4.8%	21	3.1%	14
Vomiting	2.5%	11	2.9%	13
AST increased	2.9%	13	2.2%	10
Constipation	1.4%	6	2.2%	10
Blood uric acid increased	2.0%	9	1.8%	8
Nausea	2.5%	11	1.3%	6

MedDRA/J ver.13.0

4.(iii).A.(2).2) Long-term treatment study (5.3.5.2-1: Study [redacted] [redacted] 20[redacted] to [redacted] 20[redacted])

A multi-center, open-label, uncontrolled study was conducted at 32 centers in Japan to evaluate the safety and efficacy of acotiamide and, at the same time, to investigate the pattern of treatment compliance in the long-term treatment in patients (aged ≥20 and ≤79 years) diagnosed

with FD in accordance with the Rome III criteria[67] (target sample size of ≥330).

Acotiamide (300 mg) was to be administered orally in 3 divided doses before a meal for 24 weeks. Subjects who wished to continue the treatment on and after Week 25 were evaluated for the appropriateness of continued treatment by the investigator, etc. at Week 24 and, if judged as such, were allowed to continue to take acotiamide for up to 48 weeks. At each study visit every 4 weeks, subjects were to be evaluated as to whether or not the treatment should be suspended temporarily, resumed, or discontinued according to the criteria provided in Table 44.

Table 44. Criteria for treatment suspension, resumption, etc.

[Criteria for treatment suspension] Subjects whose general impression was "feeling much better" or "feeling better" in the last 3 out of 4 study visits every 4 weeks (Week 4-44) [Criteria for resuming treatment] Subjects who met all of the following criteria at study visit of every 4 weeks (Week 4-44) - Treatment was suspended according to the criteria for treatment suspension - Subject's general impression was "feeling slightly better" at best in the last 2 out of 4 weeks during the treatment suspension according the criteria for treatment suspension - There was no safety problem in resuming the treatment, as judged by the investigator, etc. [Major criteria for discontinuation] Subjects who met the following criteria at the study visit every 4 weeks (Week 4-44) - Subject's general impression was "unchanged" at best in the last 2 out of 4 weeks during the treatment (discontinuation due to no response) - The subject did not meet the criteria for treatment resumption, with treatment suspension continuing for 12 weeks according to the criteria for treatment suspension (discontinuation due to elimination of symptoms).

Of all of 409 treated subjects, 408 subjects were included in the safety analysis set, excluding 1 subject who was enrolled in duplicate. Of these subjects, 405 subjects, excluding 3 without efficacy data, were included in the FAS for the primary efficacy analysis. The duration (median [minimum, maximum], mean ± SD) of taking the study drug in this study, excluding days of treatment suspension according to the criteria for treatment suspension, was 83.0 (4, 336) days and 102.9 ± 77.91 days, respectively.

Adverse events were observed in 72.5% (296 of 408 subjects) and adverse drug reactions in 11.5% (47 of 408 subjects). Table 45 shows adverse events observed in ≥2.0% of subjects. Adverse drug reactions observed in ≥2.0% of subjects were "constipation" and "diarrhoea," both of which occurred in 2.2% (9 of 408 subjects). There were no deaths. Serious adverse events observed were "pancreatic carcinoma, ALT increased, AST increased and blood bilirubin increased,"[68] "ALT increased," "AST increased," "meningitis bacterial" in 1 subject each. Causal relationship with the study drug was not ruled out for "ALT increased."

[67] See Table 31

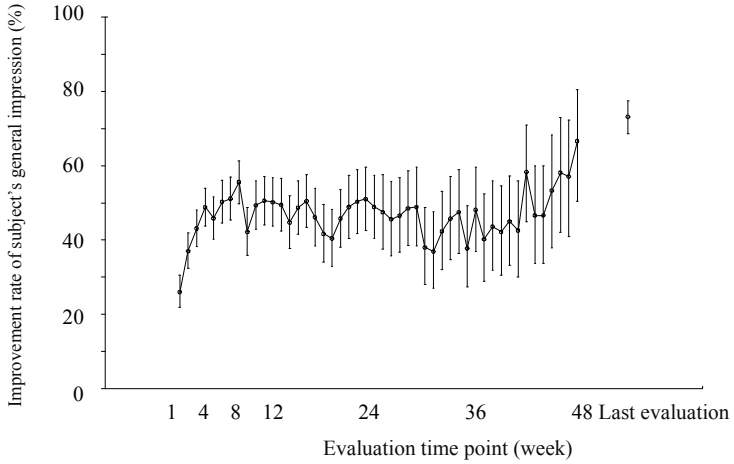
[68] This subject died of pancreatic cancer on the 93rd day after study discontinuation. It was highly likely that the cancer was present from before the study drug administration, and was judged as not causally related to the study drug.

Table 45. Adverse events observed in ≥2.0% of subjects

	Incidence	N		Incidence	N
Overall	72.5%	296	Headache	3.4%	14
Nasopharyngitis	25.7%	105	Constipation	3.2%	13
Blood triglycerides increased	24.8%	101	Pharyngitis	2.9%	12
γ-GTP increased	7.4%	30	Blood potassium increased	2.2%	9
White blood cell count increased	6.6%	27	Blood prolactin increased	2.2%	9
Diarrhoea	6.4%	26	Back pain	2.2%	9
ALT increased	6.1%	25	Blood potassium decreased	2.0%	8
Blood bilirubin increased	4.2%	17	White blood cell count decreased	2.0%	8
AST increased	3.4%	14			

MedDRA/J ver.11.0, N = 408

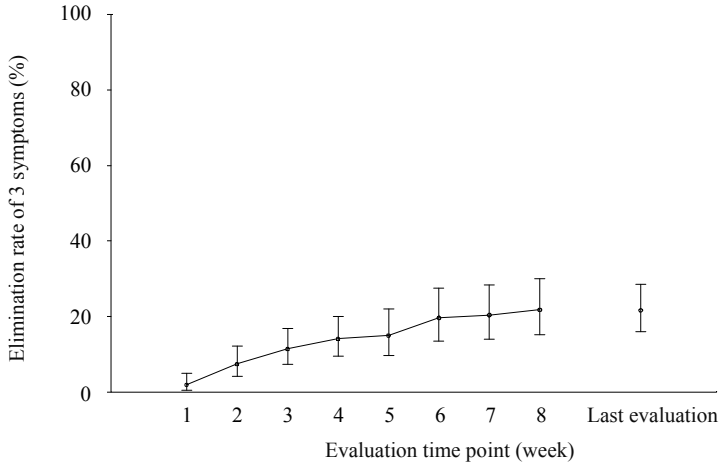
Figures 1 and 2 show the improvement rate of subject's general impression[60] (up to Week 48 and at the last evaluation time point in the treatment period) and the elimination rate of 3 symptoms[66] (up to Week 8 and at the last evaluation time point in the treatment period), respectively, at each evaluation time point.



Evaluation time point	Wk 1	Wk 4	Wk 8	Wk 12	Wk 24	Wk 36	Wk 48	Last evaluation time point
No. of subjects	403	395	302	239	141	81	42	403

Figure 1. Change over time in the improvement rate of subject's general impression (%) [95% CI]

* [Note by PMDA: In this figure, patients who discontinued treatment with acotiamide because of symptom improvement or no response were excluded from evaluation at each time point, while those who resumed taking acotiamide because of relapse after treatment suspension were included in the evaluation. Therefore, due caution should be exercised in interpreting the results.]



Evaluation time point	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Last evaluation time point
No. of subjects	405	402	401	396	319	304	303	302	405

Figure 2. Change over time in the elimination rate of 3 symptoms (%) [95% CI]

4.(iii).B Outline of the review by PMDA

4.(iii).B.(1) Functional dyspepsia

The applicant explained FD as follows:

1) Definition of the disorder

In 1987, non-ulcer dyspepsia (NUD) was proposed at the American Gastroenterological Association as the new definition of gastrointestinal disorder (*The Lancet*. 1988; 331: 576-579). NUD was defined as a syndrome with a chief complaint of chronic upper abdominal distress (e.g., upper abdominal pain, discomfort, fullness, queasy, nausea) with all primary organic diseases ruled out. However, appropriateness of the term "non-ulcer" was questioned because (1) it was unclear whether or not NUD included upper abdominal distress associated with non-ulcerative organic diseases such as cancer, causing confusion, and (2) symptoms such as nausea that were diagnosed as gastroesophageal reflux disease (GERD) was included in the above definition. Against such a background, the Rome Committee was organized following the International Congress of Gastroenterology held in 1998 in Rome with the purpose of establishing the diagnostic criteria and treatment method for "disease states that cause gastrointestinal symptoms without accompanying organic lesions." These disease states were defined as functional gastrointestinal disorders (FGID) and the criteria for classification and diagnosis were proposed as Rome I in 1994. In Rome I, a group of upper abdominal distress, excluding GERD-type symptom such as nausea, was positioned as functional dyspepsia. Subsequently, with the advancement of studies on FGID, the Rome criteria were revised to Rome II in 1999 (*Gut*. 1999;45: II37-II42), and then to Rome III in 2006 (*Gastroenterology*. 2006; 130: 1466-1479).

In Rome III, FD is defined as the presence of symptoms with one or more of the following for the last 3 months with symptom onset at least 6 months prior to diagnosis with no evidence of structural disease that is likely to explain the symptoms: bothersome postprandial fullness,[69] early satiation,[70] epigastric pain,[71] and epigastric burning.[72] Furthermore, FD is subclassified into postprandial distress syndrome (PDS) characterized by bothersome postprandial fullness or early satiation and epigastric pain syndrome (EPS) characterized by epigastric pain or epigastric burning (*Gastroenterology*. 2009;137:94-100). Overlap between PDS or EPS and GERD or irritable bowel syndrome (IBS) is considered to occur frequently.

2) Current status of clinical practice in Japan

The diagnostic criteria for FD are posted on the homepage of the Japanese Society of Gastroenterology for public access. The fact that the Phase III study was conducted in Japan in an appropriate manner suggests that FD is a disease well known among gastroenterologists and can therefore be diagnosed appropriately. In contrast, the disease is hardly well known among nonspecialist physicians because FD is not a disease name used by the health insurance plan, precluding appropriate diagnosis at the current moment. Therefore, it is important to ensure that physicians are fully informed of the diagnostic criteria and treatment method for FD. For this purpose, the applicant plans to coordinate with the Functional Dyspepsia Study Group. The applicant also plans to prepare materials for physicians to disseminate the disease concept, diagnosis, and treatment method for FD.

3) Treatment

There are no drugs approved with an indication for FD either in Japan or overseas. FD is currently treated with histamine H_2 receptor antagonists (H_2 blockers), proton pump inhibitors (PPIs), or prokinetic agents, alone or in combination. However, effects of these drugs on FD are reported variously, as described below. In these studies, FD is diagnosed using diagnostic criteria before Rome II and the study population varied from study to study, leading to inconsistent results.

It is reported that H_2 blockers and PPIs exhibit a certain efficacy in NUD patients by suppressing acid secretion (*Cochrane Database of Systematic Reviews*. 2006;4). On the other hand, it is also reported that, since NUD patients include those with chief complaint of GERD-type symptoms, efficacy of these drugs on FD patients is unclear (*Gastroenterology*. 2006;130:1466-1479), and that, although omeprazole, a PPI, was effective in patients with FD with chief complaint of ulcer-like or GERD-type symptoms fulfilling the Rome I criteria, it was no more effective than placebo in patients with chief complaint of dysmotility-like symptoms (*Aliment Pharmacol Ther*. 1998;12:1055-1065). Regarding prokinetic agents mosapride citrate hydrate (mosapride) and itopride hydrochloride (itopride), it is reported that they were no more effective than placebo in a placebo-controlled, double-blind study involving patients with FD fulfilling the Rome II criteria (*Aliment Pharmacol Ther*. 2002;16:959-967, *Gut*. 2008;57:740-746). Thus, there has been no report that validated the efficacy of existing drugs in patients with FD that fulfilled the Rome criteria.

PMDA considers as follows:

From the explanations 1) to 3) above, PMDA has confirmed the basic principle of FD, current clinical practice, and treatment method in Japan. Although FD has no effect on life prognosis,

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69 Unpleasant sensation like the prolonged persistence of food in the stomach

70 A symptom where a patient feels as if his/her stomach is overfilled soon after starting to eat a meal, and is unable to finish the meal.

71 A subjective, unpleasant sensation located at the epigastric region (region between the umbilicus and lower end of the sternum, and marked by the midclavicular lines), with or without burning sensation. Some patients may feel that tissue damage is occurring.

72 An unpleasant subjective sensation of heat in the epigastrium

decrease in QOL is a matter of concern (*Journal of the Japanese Society of Gastroenterology*. 2012;109:1683-1696) and, also in consideration of the estimated prevalence rate (*Neurogastroenterol Motil*. 2012;24:464-471, e217), it is understandable that there are substantial needs for therapeutic agents for FD. However, FD is not well known to physicians other than gastroenterologists, and existing drugs are considered to be widely used simply based on the diagnosis of gastritis, etc., without endoscopic evaluation. Since it is likely that FD is often treated by nonspecialist physicians, it is essential, for the purpose of the proper use of acotiamide, to provide appropriate information in cooperation with relevant academic societies, etc., thereby to promote the awareness of the healthcare professionals.

4.(iii).B.(2) Efficacy

As a result of the review described in 4.(iii).B.(2).1) to 4.(iii).B.(2).3) below, PMDA considers that the efficacy of acotiamide has been demonstrated. A final decision will be made taking account of comments raised in the Expert Discussion.

4.(iii).B.(2).1) Design of Phase III study

(a) Control group

Since there are currently no drugs indicated for FD, PMDA considers there is no problem in using a placebo as the control.

(b) Study patients

The Phase III study was conducted in patients with PDS as chief symptom, instead of including all FD subtypes. The applicant explained the reason for this choice as follows: When the results of 2 Phase IIb studies conducted in patients diagnosed with FD in accordance with the Rome II criteria were analyzed separately for each subgroup of the Rome III criteria (PDS and EPS), acotiamide was effective in improving postprandial fullness, upper abdominal bloating, and early satiation, among others, while the efficacy of acotiamide for EPS was unclear [see "4.(iii).B.(5).1).(a) Symptoms" for the explanation regarding the efficacy for EPS].

Regarding target patients for acotiamide, PMDA considers as follows:
There is no symptom commonly shared by all patients with FD, and the pattern of the occurrence of symptoms varies from patient to patient, suggesting that the pathology of FD is not uniform. For these reasons, the disease was subclassified into PDS and EPS in Rome III from the point of view of pathology. Therefore, it is reasonable to evaluate acotiamide by taking into account the difference in pathology between PDS and EPS.

Acotiamide is considered to enhance the movement of the gastric antrum and improve the gastric motility by inhibiting acetylcholinesterase (AChE), thereby suppressing the degradation of ACh released from cholinergic nerve terminals, resulting in enhancement of ACh-induced contraction and motility of the gastric antrum and gastric body. It is therefore understandable to expect that acotiamide is effective in treating PDS which is characterized by the symptoms possibly resulting from decreased gastric motility. In fact, the effect of acotiamide for EPS was unclear in the two Phase IIb studies.

On the basis of the above, PMDA considers it understandable to use FD patients with PDS as chief symptoms in the Phase III study.

(c) Primary endpoints

The applicant explained the reasons for selecting the primary endpoints, as follows:
The Rome III criteria recommend using an index evaluated by patients themselves for efficacy evaluation. One of the primary endpoints in the Phase III study, "subject's general impression," is an index whereby subjects evaluate the overall FD symptoms by themselves, and is therefore an appropriate index. In "subject's general impression," "feeling much better" or "better"

compared with the run-in period in the 7-grade rating scale are regarded as effective, which is a commonly used method for evaluating the treatment efficacy of drugs.

The other primary endpoint, elimination of 3 symptoms (postprandial fullness, upper abdominal bloating, early satiation), was used as an index that is more objective and strict than the subject's general impression. "Elimination of symptom" which evaluates the presence or absence of subjective symptoms varies less widely among subjects compared with the evaluation of the severity of symptoms, and only "no symptom" is judged as effective. Therefore, it is considered to be a highly objective index. The index "elimination of 3 symptoms," which judges the treatment effective only if all 3 symptoms have been eliminated, is considered to be a still more objective and strict index, compared with the index using only 1 symptom.

PMDA considers as follows:
Since FD is a disease associated only with subjective symptoms of patients, self-rating of the symptoms by patients is important. Therefore, it is appropriate to use subjects' own general impression of the disease as a primary endpoint, and to regard "feeling better or much better" as "improved" and calculate the percentage of "improved" patients. On the other hand, in order to clearly demonstrate the efficacy of a drug, it is also important to evaluate the drug using a more objective index than "subject's general impression." "Elimination of 3 symptoms," the other primary endpoint used in the Phase III study, is, although evaluated by patients themselves, considered to be a more objective index in that it requires the elimination of 3 major symptoms of PDS used as inclusion criteria in the Phase III study (postprandial fullness, upper abdominal bloating, early satiation). Since symptoms in FD patients may vary from day to day, it is acceptable that efficacy judgment was based on index values evaluated over 1 week.

Based on the above, PMDA considers it is acceptable that "subject's general impression" and "elimination of 3 symptoms" are the primary endpoints in the Phase III study.

(d) Time points for efficacy evaluation
The applicant explained the timing of evaluating the efficacy of acotiamide, as follows:
Prior to conducting clinical studies in Japan, the applicant asked gastroenterologists for their opinions. According to the results, physicians in Japan prescribe drugs usually for 14 days or 28 days, and gastroenterologists are no exception. Therefore, in the Phase IIa study conducted in FD patients for the first time in Japan, efficacy during the 28-day treatment period was investigated in an exploratory manner. Since the Phase IIa study results suggested that the 28-day treatment period was sufficient for evaluating the efficacy of acotiamide relative to placebo, the treatment period was set at 28 days for both Phase IIb1 and Phase IIb2 studies. The treatment period for the Phase III study was also set at 28 days, based on the results of the Phase IIb1 and Phase IIb2 studies and according to the Rome III recommendation that the treatment duration should be at least 28 days to investigate the periodicity of symptoms and to estimate the mechanism of action of the treatment. On the basis of the above, evaluations made at the last time point in the treatment period (Days 22-28) were used as the primary endpoints.

PMDA considers that there is no problem with the study design of the Phase III study in which the treatment period was set at 28 days and the timing of the evaluation of the primary endpoint was set at the last evaluation in the treatment period.

4.(iii).B.(2).2) Results of the primary endpoints in the Phase III study
Table 46 shows the results of the primary efficacy endpoints: "improvement rate of subject's general impression at the last evaluation time point in the treatment period" and "elimination rate of 3 symptoms at the last evaluation time point in the treatment period." Both endpoints showed a statistically significant difference between the 300 mg/day group and the placebo

group, validating the superiority of acotiamide 300 mg/day to placebo.

Table 46. Efficacy at the last evaluation time point in treatment period (FAS) (reproduced from Table 42)

	Placebo group (N = 442)	300 mg/day group (N = 450)
Subjects with improvement	154	235
Improvement rate of subject's general impression [95% CI]	34.8% [30.5%, 39.3%]	52.2% [47.6%, 56.7%]
Between-group difference of improvement rate [95% CI]	–	17.4% [11.0%, 23.7%]
P value[a)]	–	$P < 0.001$
Subjects with elimination of 3 symptoms	40	69
Elimination rate of 3 symptoms [95% CI]	9.0% [6.7%, 12.0%]	15.3% [12.2%, 18.9%]
Between-group difference for elimination rate of 3 symptoms [95% CI]	–	6.3% [2.1%, 10.5%]
P value[a)]	–	$P = 0.004$

a) Fisher's exact test, two-sided level of significance of 5%

Although statistically significant difference was observed between the 300 mg/day group and the placebo group in the two primary endpoints, the between-group difference [95% CI] for elimination rate of 3 symptoms was only 6.3% [2.1%, 10.5%]. Therefore, PMDA asked the applicant to explain the clinical significance of acotiamide by also taking account of this result.

The applicant responded as follows:
In the Phase III study, the "elimination rate of 3 symptoms at the last evaluation time point" was higher only by 6.3% in the 300 mg/day group than in the placebo group, whereas the other primary endpoint, "improvement rate of subject's general impression at the last evaluation time point", was higher by as much as 17.4% in the 300 mg/day group compared with the placebo group, showing that the difference was statistically significant for both primary endpoints. In addition, among subjects with improvement in their general impression at the last evaluation time point in the treatment period in the Phase III study, the "elimination rate of 3 symptoms" was 20.8% (32 of 154 subjects) in the placebo group and 26.4% (62 of 235 subjects) in the 300 mg/day group. Thus, only a small percentage of subjects with improved subject's general impression achieved "elimination of 3 symptoms" in both groups, indicating that "elimination of 3 symptoms" is a stricter index than the other. There have been no reports of clinical studies on FD that were conducted using "elimination of 3 symptoms" as the endpoint. Among the group of patients with FD, which shows complex symptoms with primary symptoms varying from patient to patient, a significantly higher rate of "elimination of 3 symptoms" was observed in the 300 mg/day group relative to the placebo group, indicating the treatment efficacy of acotiamide. Results of the improvement rate of subject's general impression also suggest the sufficient efficacy of acotiamide in FD patients.

PMDA considers as follows:
The Phase III study showed a statistically significant difference between the 300 mg/day group and the placebo group in "elimination rate of 3 symptoms at the last evaluation time point in the treatment period," which suggests the efficacy of acotiamide in eliminating the 3 symptoms compared with the placebo. However, since the between-group difference in the extent of the efficacy between the 300 mg/day group and the placebo group is not sufficiently large, it is difficult to clearly explain the clinical significance of acotiamide from the aspect of the elimination rate of 3 symptoms. On the other hand, since FD is a disease associated only with subjective symptoms and the goal of the drug therapy is for patients to feel the improvement of symptoms, it is of clinical significance that a statistically significant difference was observed

between the 300 mg/day group and the placebo group in "improvement rate of subject's general impression at the last evaluation time point in the treatment period" with a certain degree of between-group difference.

Thus, PMDA considers that efficacy of acotiamide has been demonstrated, based on the collective evaluation of the results of the 2 primary endpoints.

4.(iii).B.(2).3) Other endpoints in the Phase III study

(a) Improvement rate of subject's general impression and elimination rate of 3 symptoms in PPS

Table 47 shows "improvement rate of subject's general impression at the last evaluation time point in the treatment period" and "elimination rate of 3 symptoms at the last evaluation time point in the treatment period" in the per protocol set (PPS) [73] in the Phase III study (424 subjects in the placebo group, 435 subjects in the 300 mg/day group). Results were similar to those observed with FAS.

Table 47. Efficacy at the last evaluation time point in treatment period (PPS)

	Placebo group (N = 424)	300 mg/day group (N = 435)
Subjects with improvement	147	232
Improvement rate of subject's general impression [95% CI]	34.7% [30.2%, 39.3%]	53.3% [48.6%, 57.9%]
Between-group difference of improvement rate [95% CI]	–	18.7% [12.2%, 25.1%]
P value[a)]	–	$P < 0.001$
Subjects with elimination of 3 symptoms	38	66
Elimination rate of 3 symptoms [95% CI]	9.0% [6.5%, 12.0%]	15.2% [12.1%, 18.8%]
Between-group difference for elimination rate of 3 symptoms [95% CI]	–	6.2% [1.9%, 10.5%]
P value[a)]	–	$P = 0.006$

a) Fisher's exact test, two-sided level of significance of 5%

(b) Elimination rate of 3 symptoms

Table 48 shows the elimination rate of 3 symptoms at the last evaluation time point in the treatment period in the Phase III study. The elimination rate tended to be higher in the 300 mg/day group compared with the placebo group in all of these symptoms.

Table 48. Elimination rates of 3 symptoms at the last evaluation time point in the treatment period (FAS)

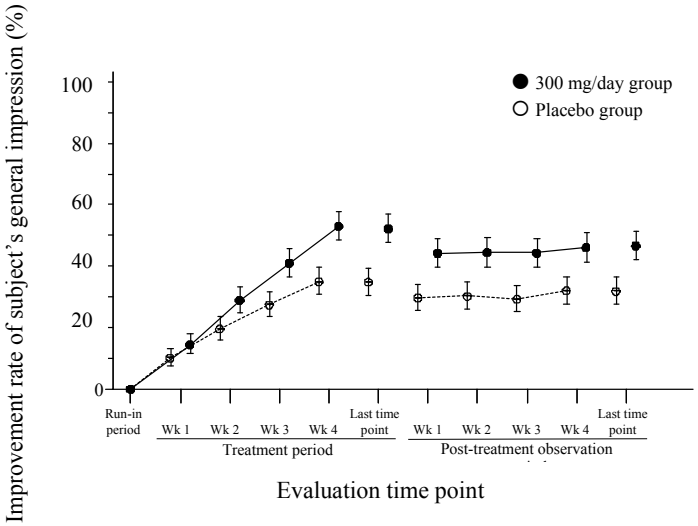
	Postprandial fullness			Upper abdominal bloating			Early satiation		
	No. of subjects analyzed	No. of subjects with elimination	Elimination rate [95% CI]	No. of subjects analyzed	No. of subjects with elimination	Elimination rate [95% CI]	No. of subjects analyzed	No. of subjects with elimination	Elimination rate [95% CI]
Placebo group (N = 442)	428	71	16.6% [13.3%, 20.4%]	376	107	28.5% [24.1%, 33.2%]	366	93	25.4% [21.2%, 30.1%]
300 mg/day group (N = 450)	444	101	22.7% [19.0%, 26.8%]	380	131	34.5% [29.8%, 39.3%]	384	145	37.8% [33.0%, 42.7%]

Patients without symptoms at the start were excluded from calculation of the elimination rate.

[73] The population of subjects in FAS, excluding those who did not meet the inclusion criteria, fell under the exclusion criteria, violated prohibited concomitant medication (except drugs prohibited only during the post-treatment observation period), or had a treatment compliance rate of <80% during the period for subjective symptom evaluation in the treatment period.

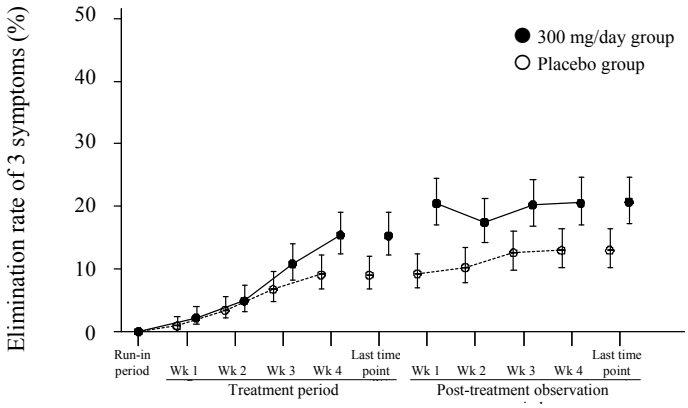
(c) Changes over time in the improvement rate of subject's general impression and in the elimination rate of 3 symptoms at each evaluation time point

In the Phase III study, the 4-week treatment period was followed by a 4-week post-treatment observation period. Figures 3 and 4 show changes over time in the "improvement rate of subject's general impression" and in the "elimination rate of 3 symptoms," respectively, at each evaluation time point including the post-treatment period. Both endpoints improved over time from Week 1 to 4, both in the placebo group and in the 300 mg/day group. The "improvement rate of subject's general impression" and the "elimination rate of 3 symptoms," tended to be higher in the 300 mg/day group compared with the placebo group, starting in Weeks 2 and 3 of the treatment period, respectively. The difference of the rates between the 2 groups remained almost unchanged from Week 1 to 4 in the post-treatment observation period.



Evaluation time point		Run-in period	Treatment period					Post-treatment observation period				
			Wk 1	Wk 2	Wk 3	Wk 4	Last	Wk 1	Wk 2	Wk 3	Wk 4	Last
No. of subjects	300 mg/day group	450	450	446	441	439	450	438	434	433	432	439
	Placebo group	442	442	440	430	428	442	430	429	427	424	430

Figure 3. Change over time in the improvement rate of subject's general impression (%) [95% CI] (FAS)



Evaluation time point		Run-in period	Treatment period					Post-treatment observation period				
			Wk 1	Wk 2	Wk 3	Wk 4	Last	Wk 1	Wk 2	Wk 3	Wk 4	Last
No. of subjects	300 mg/day group	450	450	448	443	441	450	439	435	434	433	439
	Placebo group	442	442	441	430	428	442	431	429	428	424	431

Figure 4. Change over time in the elimination rate of 3 symptoms (%) [95% CI] (FAS)

(d) QOL

During the treatment period in the Phase III study, "sum of the means of 5 domains" (mean ± SD) in SF-NDI[74] scale was 8.36 ± 3.21 in the placebo group and 7.57 ± 2.85 in the 300 mg/day group, showing a tendency of a lower score in the 300 mg/day group compared with the placebo group. Similar results were observed during the post-treatment observation period.

(e) Efficacy in long-term treatment

Figures 1 and 2 show changes over time in the improvement rate of subject's general impression (at Weeks 1-48 of treatment period, at the last evaluation time point) and the elimination rate of 3 symptoms (at Weeks 1-8 of treatment period, at the last evaluation time point), respectively, in the long-term treatment study.

Although the "improvement rate of subject's general impression" showed slight variation from Week 4 of the treatment period, that in patients who continued the study tended to remain almost at a constant level from Week 4 up to Week 48 of the treatment period. In addition, although the evaluation in the long-term treatment was difficult because it was performed only up to Week 8 of the treatment period, the elimination rate of 3 symptoms remained almost at a constant level from Week 6 to Week 8 of the treatment period and then at a similar level at the last evaluation time point.

In the long-term treatment study, since treatment was allowed to be suspended, resumed, or discontinued depending on the results of "subject's general impression" at the study visit every 4 weeks, only a small number of subjects continued to receive acotiamide until study completion. This fact should be considered [for discussion on the necessity of long-term treatment of acotiamide, see "4.(iii).B.(6).2) Treatment duration"].

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[74] A short-form of Nepean Dyspepsia Index (*Aliment Pharmacol Ther.* 2001;15:207-216, *Aliment Pharmacol Ther.* 1999;13:225-235.). This consists of a question sheet for evaluating the gastric symptoms (SF-NDI scores) and a question sheet for evaluating QOL (SF-NDI scales). SF-NDI scales are composed of 5 domains (tension caused by stomach disorder, interference with daily activities, eating/drinking, knowledge/control on stomach disorder, work/study), with each sub-scale containing 2 items (10 items in total) and being measured by a 5-point scale (the lower the value, the better the QOL).

4.(iii).B.(3) Safety

PMDA considers as follows:

The examinations described in 4.(iii).B.(3).1) to 4.(iii).B.(3).3) below showed the findings that the incidence of adverse events was similar between the acotiamide group and the placebo group, that there were only a few severe events, and that there were no adverse events that occurred at a higher incidence with the long-term treatment. Therefore, the safety of acotiamide is acceptable, based on the results of the clinical data submitted.

The safety of acotiamide will be finalized, taking account of comments raised in the Expert Discussion.

4.(iii).B.(3).1) Comparison with the placebo group

In order to compare the safety between acotiamide and placebo, results obtained from 5 placebo-controlled studies (Phase IIa, Phase IIb1, Phase IIb2, Phase III, ultrasonography studies) were combined and subjected to analysis. Table 49 shows adverse events that were observed in ≥2.0% of subjects in any group. The occurrence of adverse events was similar between the placebo group and each of the acotiamide groups, with no particular increase in the incidence being observed after acotiamide administration.

Table 49. Adverse events observed in ≥2.0% of subjects in any group (results of 5 studies combined)

	Placebo group (N = 714)		150 mg/day group (N = 148)		300 mg/day group (N = 717)		900 mg/day group (N = 257)	
	Incidence	N	Incidence	N	Incidence	N	Incidence	N
Overall	57.0%	407	57.4%	85	55.8%	400	50.2%	129
Blood triglycerides increased	16.9%	121	12.8%	19	15.6%	112	10.5%	27
Nasopharyngitis	7.0%	50	6.1%	9	7.5%	54	4.7%	12
Blood prolactin increased	5.7%	41	4.1%	6	6.0%	43	5.1%	13
γ-GTP increased	5.2%	37	2.0%	3	5.7%	41	3.1%	8
Diarrhoea	4.3%	31	2.7%	4	5.4%	39	4.3%	11
Blood bilirubin increased	3.4%	24	2.0%	3	4.6%	33	3.1%	8
ALT increased	3.2%	23	2.7%	4	3.9%	28	1.2%	3
Dyspepsia	3.1%	22	3.4%	5	3.2%	23	1.2%	3
Vomiting	2.2%	16	2.7%	4	3.1%	22	1.9%	5
White blood cell count increased	3.4%	24	2.0%	3	2.6%	19	1.6%	4
Nausea	3.1%	22	2.0%	3	2.2%	16	1.2%	3
Constipation	1.1%	8	2.7%	4	2.0%	14	0.4%	1
AST increased	2.2%	16	2.0%	3	2.0%	14	0.8%	2
Headache	2.2%	16	2.0%	3	1.7%	12	2.7%	7
Platelet count increased	0.3%	2	2.0%	3	1.3%	9	0.0%	0
Eructation	1.3%	9	2.0%	3	1.1%	8	0.8%	2
Abdominal pain upper	1.3%	9	1.4%	2	1.0%	7	2.7%	7
Somnolence	0.7%	5	2.0%	3	0.6%	4	1.6%	4
Dysmenorrhoea	1.4%	10	4.1%	6	0.4%	3	1.6%	4

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4.(iii).B.(3).2) Serious adverse events and adverse events leading to study drug discontinuation

Table 50 shows serious adverse events and adverse events that resulted in study drug discontinuation in the 6 Japanese clinical studies in FD patients, consisting of the 5 placebo-controlled studies (Phase IIa, Phase IIb1, Phase IIb2, Phase III, ultrasonography studies) and the long-term treatment study.

Table 50. Serious adverse events and adverse events leading to study drug discontinuation in the Japanese clinical studies in FD patients

Type of adverse events	Clinical study	Treatment group	N	Adverse events
Serious adverse events	Phase IIa study	300 mg/day group	1	Asthma
	Phase III study	300 mg/day group	1	Intervertebral disc disorder
	Long-term treatment study	300 mg/day group	4	ALT increased, AST increased, blood bilirubin increased, and pancreatic carcinoma; ALT increased; AST increased; meningitis bacterial
Adverse events leading to study drug discontinuation	Phase IIb1 study	Placebo group	2	Breast disorder, dysgeusia
		300 mg/day group	2	Abdominal distension and dizziness; menopausal symptoms
		900 mg/day group	4	Rash (2 subjects); rash pruritic and diarrhoea; acute tonsillitis
	Phase IIb2 study	150 mg/day group	2	Abdominal pain upper, eczema nummular, cluster headache
		300 mg/day group	1	Pyrexia, acute tonsillitis, malaise, arthralgia, and headache
		900 mg/day group	3	Breast swelling and menorrhagia; diarrhoea; salpingo-oophoritis; gastroenteritis
	Phase III study	Placebo group	3	Haemorrhoids, sudden deafness, rash
		300 mg/day group	3	Vomiting and diarrhoea; diarrhoea; dyspepsia
	Long-term treatment study	300 mg/day group	17	Diarrhoea (2 subjects); nausea (2 subjects); pancreatic carcinoma, AST increased, ALT increased, blood bilirubin increased, blood LDH increased, blood ALP increased, and γ-GTP increased; constipation; acute sinusitis; meningitis bacterial; pharyngitis; sinusitis; enteritis infectious; AST increased; blood triglycerides increased; back pain; sciatica; urticaria; hyperthyroidism

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In the ultrasonography study, there were no serious adverse events or adverse events leading to study drug discontinuation.

One patient in the long-term treatment study died of "pancreatic carcinoma" on the 93rd day after study discontinuation. The causal relationship of the death with the study drug was ruled out. Serious adverse events were observed only in subjects in the 300 mg/day group, but their causal relationship with the study drug was ruled out except "ALT increased" in 1 subject in the long-term treatment study. In the Japanese clinical studies in FD patients, adverse events leading to study drug discontinuation in at least 2 subjects in the acotiamide groups were "diarrhoea" (6 subjects), "rash," "nausea," "acute tonsillitis," and "AST increased" (2 subjects each). Among them, adverse events for which causal relationship with the study drug could not be ruled out were "diarrhoea" in 4 subjects and "nausea" and "rash" in 2 subjects each. There was no tendency for any particular adverse event becoming serious. Similarly, adverse events leading to study drug discontinuation did not show any particular trend although "diarrhoea" was observed at a slightly higher frequency.

4.(iii).B.(3).3) Occurrence of adverse events by time period in long-term treatment study

Table 51 shows the occurrence, by time period, of adverse events with an incidence of ≥2.0% in the long-term treatment study.

Table 51. Occurrence, by time period, of adverse events with an incidence of ≥2.0% in the long-term treatment study

	- week 12 (N = 408)		Week 13-24 (N = 329)		Week 25-36 (N = 172)		Week 37-48 (N = 102)		Week 48 - (N = 46)		Entire period (N = 408)	
	Inci-dence	N	Inci-dence	N	Inci-dence	N	Inci-dence	N	Inci-dence	N	Inci-dence	N
Overall	55.9%	228	37.7%	124	29.7%	51	33.3%	34	21.7%	10	72.5%	296
Nasopharyngitis	19.4%	79	9.7%	32	4.7%	8	3.9%	4	0.0%	0	25.7%	105
Blood triglycerides increased	14.7%	60	8.2%	27	8.1%	14	6.9%	7	6.5%	3	24.8%	101
γ-GTP increased	4.4%	18	1.5%	5	2.9%	5	2.0%	2	2.2%	1	7.4%	30
White blood cell count increased	3.9%	16	1.5%	5	1.2%	2	2.9%	3	2.2%	1	6.6%	27
Diarrhoea	3.7%	15	2.1%	7	2.9%	5	2.0%	2	0.0%	0	6.4%	26
ALT increased	2.7%	11	3.0%	10	2.3%	4	2.0%	2	0.0%	0	6.1%	25
Blood bilirubin increased	2.7%	11	1.5%	5	2.9%	5	0.0%	0	0.0%	0	4.2%	17
AST increased	2.2%	9	0.9%	3	1.7%	3	1.0%	1	0.0%	0	3.4%	14
Headache	2.2%	9	0.9%	3	1.2%	2	2.9%	3	2.2%	1	3.4%	14
Constipation	2.2%	9	1.2%	4	0.6%	1	1.0%	1	0.0%	0	3.2%	13
Pharyngitis	2.5%	10	0.3%	1	1.2%	2	2.9%	3	0.0%	0	2.9%	12
Blood potassium increased	1.0%	4	1.2%	4	0.6%	1	0.0%	0	0.0%	0	2.2%	9
Blood prolactin increased	1.5%	6	0.6%	2	1.2%	2	0.0%	0	0.0%	0	2.2%	9
Back pain	2.0%	8	0.3%	1	0.0%	0	0.0%	0	0.0%	0	2.2%	9
Blood potassium decreased	0.5%	2	1.2%	4	1.2%	2	0.0%	0	0.0%	0	2.0%	8
White blood cell count decreased	0.7%	3	0.9%	3	0.6%	1	0.0%	0	2.2%	1	2.0%	8

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Although it should be noted that only a limited number continued to receive the study drug [see "4.(iii).B.(6).2) Treatment duration"], there was no tendency of adverse events occurring in an increased frequency or of any new adverse event emerging.

4.(iii).B.(4) Clinical positioning of acotiamide

The applicant explained the clinical positioning of acotiamide as follows:

Although there has been no study report that validated the efficacy of any drug in patients diagnosed with FD in accordance with the Rome criteria, based on the current situation that prokinetic agents are recommended for the treatment of PDS (*J Gastroenterology*. 2008;43:251-255), [redacted] were extracted for mosapride, itopride, and domperidone, which ranked top 3 in sales among prokinetic agents approved in Japan, according to a report by IMS Japan K.K. in 2009. As a result, relevant studies extracted were 1 foreign study on mosapride (*Aliment Pharmacol Ther*. 2002;16:959-967) and 1 foreign study on itopride (*Gut*. 2008;57:740-746). No relevant study on domperidone could be confirmed as far as this survey was concerned. Because of the limited extent of information available from the published studies on mosapride and itopride, a precise comparison was difficult. These studies were conducted in patients with FD meeting the Rome II criteria. Since there are no significant differences in the patient population between the Rome II and Rome III criteria and patients with reflux oesophagitis or irritable bowel syndrome, organic diseases, were excluded from these studies, the study population in each of these studies is considered to be similar to that in studies on acotiamide.

Regarding efficacy, the mosapride study did not show any difference between the placebo group

and the mosapride groups in the primary endpoint-change in "symptom score based on patient diary" at Week 6 from baseline. Similarly, the itopride study did not detect any difference between the placebo group and the itopride group in the "rate of improvement in patient's evaluation" after 8 weeks. There were no studies that used the elimination rate of 3 symptoms as the endpoint for any drug. Regarding safety, the incidence of adverse events was not published either in the mosapride study or in the itopride study; but was reported to be comparable between the active drug group and the placebo group.

Thus, there was little or no difference in efficacy between the mosapride or itopride group and the placebo group, whereas acotiamide was shown to be superior to placebo in the Phase III study. In addition, acotiamide, mosapride, and itopride are all comparable to placebo in the incidence of adverse events, suggesting a high safety profile of acotiamide.

PMDA considers as follows:
Although the disease name FD was advocated only in recent years, patients with upper abdominal symptoms have been prescribed with existing drugs under the diagnosis of gastritis, etc. Because no studies for direct comparison of acotiamide and existing drugs have been conducted, a definite evaluation cannot be made on the efficacy of acotiamide relative to other drugs. However, given that acotiamide was demonstrated to be effective in the Phase III study which was conducted in FD patients mainly with PDS symptoms and that acotiamide showed no clinically significant adverse events compared with placebo, acotiamide is expected to be useful as a new therapeutic agent for FD patients with symptoms of PDS. However, because of scant information available at the current moment, the clinical positioning and the choice between acotiamide and existing drugs will become clear with the accumulation of use experience in medical practice in the future.

4.(iii).B.(5) Indications

PMDA reviewed the indications of acotiamide as described in 4.(iii).B.(5).1) and 4.(iii).B.(5).2) below. The indications will be finalized taking account of comments raised in the Expert Discussion.

4.(iii).B.(5).1) Target patients

(a) Symptoms

Among the clinical studies of acotiamide, the Phase III study and the long-term treatment study were conducted in FD patients with PDS as defined in the Rome III criteria. Therefore, PMDA asked the opinion of the applicant on the lack of evidence on the efficacy and safety of acotiamide in FD patients with EPS.

The applicant responded as follows:
In the Phase IIb1 and Phase IIb2 studies, results of "improvement rate of subject's general impression at the last evaluation time point," separately calculated for patients with PDS and those with EPS, are as shown in Table 52.

Table 52. Improvement rate of subject's general impression at the last evaluation time point by disease subtype (PDS and EPS) (FAS)

		Placebo group	150 mg/day group	300 mg/day group	900 mg/day group
Phase IIb1 study	PDS subjects	40.6% (13/32)		55.2% (16/29)	55.9% (19/34)
	EPS subjects	46.4% (26/56)		48.3% (28/58)	43.1% (25/58)
Phase IIb2 study	PDS subjects	43.8% (32/73)	47.4% (37/78)	62.9% (44/70)	57.9% (44/76)
	EPS subjects	58.3% (21/36)	51.4% (19/37)	50.0% (16/32)	52.6% (20/38)

The late phase II studies were conducted based on the Rome II criteria and data were not collected based on the subclassification of the Rome III criteria. Therefore, patients with chief complaint of upper abdominal pain or upper abdominal discomfort were defined as EPS patients, and those with chief complaint of postprandial fullness, upper abdominal bloating, or early satiation as PDS patients.

The efficacy of acotiamide in EPS patients was unclear from the results of these studies.

Regarding safety, in contrast, results of the combined analysis of the Phase IIb1, Phase IIb2, ultrasonography, Phase III, and long-term treatment studies showed that the incidence of adverse events by disease type in the acotiamide groups combined (150, 300, 900 mg/day groups) was similar between PDS patients (60.8% [705 of 1160 patients]) and EPS patients (53.7% [130 of 242 patients]). In addition, the incidence of individual adverse events was also similar between PDS patients and EPS patients. These results suggest that administration of acotiamide in EPS patients does not pose any safety concern.

On the basis of the above, the applicant considered that, although there is no clear evidence to support the efficacy of acotiamide in EPS patients, cautions have already been included in the proposed indications "functional dyspepsia (gastrointestinal symptoms such as postprandial fullness, upper abdominal bloating, or early satiation)" which clearly represents the characteristic feature of acotiamide.

PMDA considers as follows:
The indications should be appropriately determined in line with the eligible patients and the results of the clinical studies. Therefore, based on the results of the Phase III study that demonstrated the efficacy of acotiamide in FD patients mainly with PDS as defined in the Rome III criteria, the indications should be set in such a way as to explicitly indicate the usefulness for treating postprandial fullness, upper abdominal bloating, and early satiation among symptoms of FD. In addition, information should be provided that results of the clinical studies have not shown the efficacy of acotiamide in FD patients mainly with EPS as defined in the Rome III criteria.

(b) Symptomatic period
Rome III criteria specify that symptoms should have lasted for the last 3 months or longer with the onset at least 6 months prior to diagnosis. In Japan, compared with situations in other countries, it is easier for patients to receive care, including endoscopy, at medical institutions. Therefore, patients are more likely to visit medical institutions within 6 months after the onset of symptoms. PMDA considers it necessary to determine the eligible patients appropriately in the clinical studies in patients with FD by specifying the symptomatic period, etc. in accordance with the Rome III criteria. At the same time, however, PMDA considers that, in clinical practice, the symptomatic period and the duration of symptoms need not strictly follow the Rome III criteria. Instead, treatment should be given in a flexible manner based on the status of the actual clinical practice in Japan.

4.(iii).B.(5).2) Exclusion of organic diseases, etc.
Since patients with organic diseases, etc., should be excluded from diagnosis of FD, PMDA asked the applicant to explain the method for appropriately excluding, before administering acotiamide, organic diseases including peptic ulcer and malignant diseases such as gastric cancer which occur in Japanese patients relatively frequently.

The applicant responded as follows:
In the Phase III study, organic diseases were detected and the study was discontinued during the run-in period in 14.4% (200 of 1393 patients) among patients who had met the criteria for FD at the interview. Organic diseases detected were, in the order of decreasing frequency, erosion, reflux oesophagitis, esophageal hiatus hernia, and ulcer. On the other hand, in the Japan Mosapride Mega Study (JMMS) which investigated the efficacy and safety of mosapride in FD patients (*Clinical practice*. 2007; 26: 397-400), organic diseases were detected in 9% (90 of 1027 subjects), with reflux oesophagitis, esophageal hiatus hernia, peptic ulcer, and erosive gastritis being observed frequently. Malignant diseases were detected in 0.07% (1 of 1393

patients) in the Phase III study and in 0.3% (3 of 1027 patients) in JMMS. Thus, the incidence of malignant diseases was similar to that observed in cancer screening (0.05%-0.27%, according to the Report on Regional Public Health Services and Health Services for the Aged, MHLW, 2007). These results suggest that there may be a risk that 10 % of organic diseases including malignant diseases are left undetected by patient interview. Highly specialized tests are necessary to completely exclude patients with these diseases. However, since there are many patients with upper abdominal distress, it is unrealistic to require all patients suspected of FD by interview to undergo specialized tests. Furthermore, it poses an ethical problem to force all of these patients to receive invasive specialized tests because of the physical and psychological burdens placed on them. Therefore, the applicant considered it appropriate to provide a caution in the package insert that a specialized test should be performed as appropriate if symptoms do not improve after a certain period of treatment with acotiamide.

PMDA considers as follows:
It is reasonable to perform appropriate specialized tests instead of continuing the treatment with acotiamide without careful consideration when symptoms do not improve after the treatment. At the same time, caution should be raised so that a thorough interview and test is performed before acotiamide administration and patients with organic diseases are excluded appropriately by upper gastrointestinal endoscopy, etc.

4.(iii).B.(6) Dosage and Administration
PMDA reviewed the dosage and administration as described in 4.(iii).B.(6).1) and 4.(iii).B.(6).2) below. The dosage and administration will be finalized taking account of comments raised in the Expert Discussion.

4.(iii).B.(6).1) Recommended dosage and administration
The applicant explained the justification for the dosage regimen of acotiamide as follows:
In the Phase IIa study, acotiamide was administered orally before a meal three times daily at the daily dose of 150, 300, or 900 mg. Results suggested the efficacy in the 300 mg/day group and 900 mg/day group, and there was no significant difference in the incidence of adverse events between the dose groups. Therefore, the Phase IIb1 study was conducted with daily doses of 300 and 900 mg. As a result, the "improvement rate of subject's general impression at the last evaluation time point," a primary efficacy endpoint, was 41.7% (43 of 103 subjects) in the placebo group, 51.5% (52 of 101 subjects) in the 300 mg/day group, and 49.5% (50 of 101 subjects) in the 900 mg/day group, with the rate in the 300 mg/day group being 9.8% higher than that in the placebo group (Table 36). There was no significant difference in the incidence of adverse events among the dose groups (Table 37). From these results, the recommended daily clinical dose was estimated to be 300 mg. However, based on the results of [redacted] consultation (PMDA/CPE Notification No. 0218008, of the Center for Product Evaluation, PMDA, dated [redacted]), the dose-response investigation in the Phase IIa study was considered to be insufficient. Therefore, dose-response of acotiamide was further investigated in the Phase IIb2 study which was conducted in the 4 treatment groups, 3 active drug groups centered around 300 mg/day, the estimated recommended clinical dose, plus the placebo group (i.e., 150, 300, 900 mg/day groups, and the placebo group). As a result, the "improvement rate of subject's general impression at the last evaluation time point," a primary efficacy endpoint, was 49.1% (55 of 112 subjects) in the placebo group, 48.7% (56 of 115 subjects) in the 150 mg/day group, 58.3% (63 of 108 subjects) in the 300 mg/day group, and 56.9% (66 of 116 subjects) in the 900 mg/day group, with the rate in the 300 mg/day group being 9.2% higher than in the placebo group. However, no statistically significant dose-response relationship was observed (Table 39). There was no significant difference in the incidence of adverse events among the dose groups. Thus, although no statistically significant dose-response relationship was observed either in the Phase IIb1 study or in the Phase IIb2 study, the improvement rate was the highest in the 300 mg/day group and an approximately 10% higher

rate relative to the placebo group was observed in a reproducible manner. Therefore, the recommended daily clinical dose was estimated to be 300 mg, and the Phase III study was conducted using this daily dose. Results verified the superiority of the acotiamide 300 mg/day group to the placebo group in both primary efficacy endpoints, "improvement rate of subject's general impression at the last evaluation time point in the treatment period" and "elimination rate of 3 symptoms at the last evaluation time point in the treatment period." Also, adverse events in the 300 mg/day group did not pose any significant clinical concerns relative to those in the placebo group.

On the basis of the above results, the applicant set the dosage regimen for acotiamide; 100 mg to be administered orally three times daily before a meal (300 mg/day).

PMDA considers as follows:
Although no clear dose-response relationship was observed for acotiamide in each of the Phase II studies, the results of subpopulation analysis performed separately in PDS and EPS patients suggests that efficacy in the 300 mg/day group of PDS patients tended to be higher (Table 52). Therefore, the daily dose of acotiamide in the Phase III study was set at 300 mg, based on the results of Phase IIb1 and Phase IIb2 studies, which is understandable. Results demonstrated the efficacy of acotiamide in the 300 mg/day group with no clinically significant safety problems relative to the placebo group. Thus, it is acceptable to set the daily dose of acotiamide at 300 mg. Also, since symptoms of PDS are related to food consumption, it is understandable to administer the drug before a meal.

4.(iii).B.(6).2) Treatment duration

PMDA asked the applicant to explain the necessity of long-term treatment and the appropriate treatment duration, based on the results of the long-term treatment study.

The applicant responded as follows:
In the long-term treatment study, the improvement rate of subject's general impression was 26.1% (105 of 403 subjects) at Week 1 of the treatment period, 37.1% (149 of 402 subjects) at Week 2, 43.1% (173 of 401 subjects) at Week 3, and 48.9% (193 of 395 subjects) at Week 4, showing a continuous increase from the start of the treatment up to Week 4 of the treatment period (Figure 1). From Week 5, subjects were to take the drug according to the criteria shown in Table 44. As a result, administration was suspended temporarily or discontinued in a greater number of subjects than expected. To compensate for these subjects, a total of 412 subjects, far exceeding the target sample size (330 subjects), were enrolled in the study. Of 405 subjects included in FAS, 107 subjects were treated with acotiamide for >24 weeks, including those with treatment suspension/resumption; 53 subjects for >44 and ≤48 weeks; and 5 subjects for 48 consecutive weeks. Treatment was suspended temporarily in 75.1% (304 of 405 patients), discontinued because of elimination of symptoms in 38.0% (154 of 405 subjects), and discontinued because of no response in 13.6% (55 of 405 subjects), obviating the need for long-term treatment in many subjects.

Further investigation on the timing of treatment discontinuation showed that treatment was suspended because of sufficient response in 27.7% (112 of 405 subjects) in FAS at the study visit at Week 4 after the start of treatment. In the phase II studies (Phase IIa, Phase IIb1, Phase IIb2) and the phase III study, the improvement rate of subject's general impression increased consistently from the start of treatment up to Week 4 of the treatment period. Furthermore, in patients judged as "improved" at Week 4 of the treatment period in the Phase III study, improvement was observed almost uniformly from Weeks 1 to 4 of the treatment period. These results suggest that response to acotiamide should be monitored for at least 1 month after treatment start.

In addition, of 55 subjects in whom treatment was discontinued because of no response, a majority (56.4% [31 of 55 subjects]) discontinued the study at Week 4, which suggested that treatment with acotiamide should not be continued without careful consideration in subjects who do not show improvement in symptoms.

On the basis of the above results, the applicant considered that it is appropriate to determine the necessity of continued treatment at around 1 month after treatment start. Also, it is necessary to raise caution not to aimlessly continue administering acotiamide to patients without improvement.

The appropriate treatment duration was verified using the results from the long-term treatment study. Among patients in whom treatment was suspended (75.1% [304 of 405 patients]) according to the criteria for treatment suspension, 50.7% (154 of 304 patients) discontinued the treatment eventually because of the elimination of symptoms, obviating the necessity of treatment resumption. More specifically, treatment was suspended in those patients since they showed improvement during the last 3 weeks at study visits of every 4 weeks (treatment suspension according to the criteria for treatment suspension), and treatment was discontinued because of the continued disappearance of symptoms even after 12-week treatment suspension. This suggests that symptoms do not relapse for at least 12 weeks in approximately half of patients who showed improvement in subject's general impression continuously for 3 weeks or longer. In FAS, the mean length until the first treatment suspension was 60.4 days, whereas the first treatment suspension occurred at Week 4 of the treatment period in 27.7% (112 of 405 patients), at Week 8 in 26.7% (108 of 405 patients), and at Week 12 in 11.4% (46 of 405 patients), showing a certain number of patients withdrawn from treatment at all time points, thereby making it difficult to specify the timing for deciding treatment suspension. Thus, based on the results of the above study, the applicant considered it appropriate to give consideration to treatment suspension once when symptoms are improved continuously for a certain period of time of at least 3 weeks.

Therefore, the package insert will include the following caution statement: acotiamide should not be administered without careful consideration when no improvement is observed for a certain period (usually 1 month) after the start of treatment, and consideration should be given to treatment suspension if improvement in symptoms is observed continuously.

PMDA considers as follows:
In the long-term treatment study which set the criteria for treatment suspension or discontinuation due to elimination of symptoms and for discontinuation because of no response, most of the patients met the criteria for treatment suspension or discontinuation, whereas, of the 405 evaluable patients, only 5 were continuously treated with acotiamide for 48 weeks; this suggests that only a very limited number of patients will need long-term treatment with acotiamide. Therefore, as suggested by the applicant, caution should be raised not to continue treatment with acotiamide without careful consideration if symptoms remain improved over several weeks or if symptoms do not improve after treatment for approximately 1 month.

One patient died of pancreatic carcinoma in the long-term treatment study. The patient had been suffering from FD for more than 1 year and upper gastrointestinal endoscopy before acotiamide administration detected no abnormality. On Day 208 after acotiamide administration was started, the patient was diagnosed with pancreatic carcinoma (stage IV) at another hospital. Differential diagnosis from organic diseases is important in the treatment of FD, and it should be borne in mind that upper gastrointestinal endoscopy alone is difficult to make accurate diagnosis, as was the case with this subject. It is therefore necessary that patients be monitored for their conditions

even after the start of treatment with acotiamide, or any other drugs for that matter, and additional measures such as ultrasonography or other tests should be taken as necessary.

4.(iii).B.(7) Concomitant use with other drugs

The applicant explained concomitant use of acotiamide with drugs that may be used for the treatment of FD, as follows:

According to the FD treatment algorithm following Rome III, the first-line drugs are prokinetic agents for PDS and acid secretion inhibitors for EPS. It is advocated that acid secretion inhibitors should be added or substituted for prokinetic agents if PDS does not respond to prokinetic agents, and prokinetic agents should be added or substituted for acid secretion inhibitors if EPS does not respond to acid secretion inhibitors. Therefore, acotiamide may be used concomitantly with acid secretion inhibitors. Furthermore, considering the characteristics of FD that symptoms vary from patient to patient, drugs other than acid secretion inhibitors may be used concomitantly with acotiamide, including, for example, antispasmodic drugs when pain is severe, traditional Chinese medicines when acotiamide is not sufficiently effective, and anxiolytic agents or antidepressants if psychiatric disorder is suspected as a cause of the symptoms.

In the clinical studies of acotiamide, however, a certain restriction had been placed on the combination of some types of drugs to accurately assess the efficacy of acotiamide and, as a result, concomitant use of the above drugs was prohibited.

For these reasons, no sufficient information is currently available on the safety and efficacy of acotiamide in concomitant use with other drugs. Therefore, the applicant plans to evaluate the effect of concomitant drugs on the safety and efficacy of acotiamide in routine use via post-marketing surveillance.

Since acotiamide inhibits AChE, when acotiamide is concomitantly administered with cholinergic agents or other AChE inhibitors, the efficacy of these drugs may be enhanced, or inversely, concomitant use of acotiamide with anti-cholinergic agents may attenuate their effects. Cautions will be raised in the Interactions section of the package insert.

PMDA considers as follows:

Since patients with FD have different symptoms and backgrounds from one another, they may take various drugs concomitantly when symptoms are not improved by acotiamide alone. Therefore, it is necessary to provide appropriate cautions on drugs that require careful concomitant use and to collect information on drugs concomitantly administered via post-marketing surveillance, etc.

4.(iii).B.(8) Special populations

4.(iii).B.(8).1) Elderly patients

Each of the Phase II studies enrolled patients aged ≤79 years, whereas the Phase III study enrolled patients aged ≤64 years. PMDA asked the applicant to explain the reason for the different age settings and to explain the efficacy and safety of acotiamide in elderly patients.

The applicant responded as follows:

The percentage of FD patients aged ≥65 years enrolled in each of the Phase II studies was as follows: 5.5% (7 of 127 subjects) in the Phase IIa study, 3.1% (10 of 322 subjects) in the Phase IIb1 study, and 6.1% (28 of 461 subjects) in the Phase IIb2 study. In the Phase IIb2 study with 28 elderly subjects enrolled, the incidence of adverse events in the acotiamide group was 55.5% (177 of 319 subjects) in the non-elderly subgroup (20-64 years) and 39.1% (9 of 23 subjects) in the elderly subgroup (≥65 years). The incidence of adverse events in the elderly patients did not

tend to increase. However, physiological functions such as renal and hepatic functions are generally likely to decrease with age and, with consideration given to the effect of these reduced physiological functions on safety, the Phase III study was conducted in the non-elderly who constitute the major patient population. However, since acotiamide, once approved, is expected to be used for elderly patients as well, the long-term treatment study enrolled elderly patients also to investigate the safety in this age group.

Results of the combined analysis of the Japanese clinical studies on FD patients (Phase IIa, Phase IIb1, Phase IIb2, Phase III, long-term treatment, ultrasonography studies) showed that the incidence of adverse events was similar between the non-elderly and elderly populations, at 59.6% (877 of 1472 subjects) and 56.9% (33 of 58 subjects), respectively. Regarding the efficacy, the "improvement rate of subject's general impression at the last evaluation time point in the treatment period" in elderly subjects was 40.0% (2 of 5 subjects) in the placebo group, 66.7% (4 of 6 subjects) in the 150 mg/day group, 28.6% (2 of 7 subjects) in the 300 mg/day group, and 50.0% (5 of 10 subjects) in the 900 mg/day group in the Phase IIb2 study (including 28 elderly subjects); and 76.2% (16 of 21 subjects) in the 300 mg/day group in the long-term treatment study (including 21 elderly subjects).

Because of the very limited number of elderly subjects so far studied, the applicant plans to investigate the safety and efficacy in the elderly and non-elderly populations via post-marketing surveillance.

PMDA confirmed the background and reason for enrolling patients aged ≤64 years in the Phase III study only and the currently available information related to the safety and efficacy of acotiamide in the elderly patients. As explained by the applicant, given the limited number of elderly patients investigated in the clinical studies, it is necessary to collect, in an appropriate manner, information related to the safety and efficacy in elderly patients via post-marketing surveillance as well.

4.(iii).B.(8).2) Pregnant or nursing women

Clinical studies were not conducted on the use during pregnancy or lactation. However, there were 5 pregnant women (all in the placebo group) in the Phase III study, 3 in the long-term treatment study, and 1 in the foreign TQT study.

Of the 3 pregnant subjects in the long-term treatment study, 1 subject opted for induced abortion due to personal reasons although no abnormality was found either in the fetus or in the maternal body, while the remaining 2 subjects gave birth to normal babies. In 1 subject in the foreign TQT study (900 mg acotiamide administered 3 times daily), the last dose was given on Day 5 after the start of treatment and pregnancy was found on Day 10, whereupon the study was discontinued. Induced abortion was performed for the subject's personal reasons.

PMDA considers as follows:

Given that (1) there is scanty information on the safety of acotiamide in pregnant women; (2) in the clinical studies, blood prolactin increased was observed as a major adverse event after acotiamide administration, albeit at a similar incidence as that in the placebo group; and (3) in the non-clinical studies, radioactivity was excreted into breast milk following a single oral dose of ^{14}C-labeled acotiamide (10 mg/kg) [see "3.(ii).A.(4).3) Excretion into breast milk"], it is appropriate to include in the package insert a caution statement that safety in pregnant women has not been established and that acotiamide administration should be avoided during lactation. If it is found that acotiamide has been used in pregnant patients or that pregnancy is found during treatment with acotiamide via post-marketing surveillance, such cases should be investigated in detail.

4.(iii).B.(9) Post-marketing surveillance, etc.

In order to collect information on the safety and efficacy of acotiamide in routine use, the applicant plans to conduct post-marketing surveillance as shown in Table 53 below.

Table 53. Outline of use-results survey (draft)

Objectives	To investigate (i) unexpected adverse drug reactions, (ii) occurrence of adverse drug reactions in routine use, and (iii) factors affecting the safety and efficacy
Survey method	Central registration system
Patients surveyed	Patients for whom acotiamide is indicated
Survey duration	From post-approval until the end of observation for the planned number of patients, with a 4-week survey period in each patient
Planned number of patients	3000
Main survey items	- Patient background characteristics (age, sex, medical history, complications, pregnancy, visit category, history of allergy, smoking habit, disease duration, whether endoscopy was performed or not, chief complaint, etc.) - Use status of acotiamide - Concomitant drugs or therapies - Presence or absence of *H. Pylori* infection - Adverse events that occurred during or after treatment with acotiamide - Overall evaluation of the efficacy of acotiamide (overall improvement assessed by physician, patient's general impression, 3 major symptoms [postprandial fullness, upper abdominal bloating, early satiation], other subjective symptoms, etc.)

PMDA considers that the applicant should collect and review the following information which is unavailable in sufficient detail in the data of clinical studies, via post-marketing surveillance.

- Safety in elderly patients, patient with renal impairment, and patients with hepatic function disorder
- Clinical course during treatment with acotiamide and relapse of symptoms
- Presence or absence of other concurrent diseases such as GERD and IBS, and safety and efficacy in patients with concurrent diseases
- Safety and efficacy by presence or absence of *H. Pylori* infection
- Occurrence of events induced by cholinergic effect
- Safety of acotiamide when concomitantly administered with a cholinergic agent or an anti-cholinergic agent
- Effect of concurrent psychiatric disorders such as depression and sleep disorder

A final decision on the details of post-marketing surveillance, including items to be investigated, will be made, taking account of comments raised in the Expert Discussion.

III. Results of Compliance Assessment Concerning the Data Submitted in the New Drug Application and Conclusion by PMDA

1. PMDA's conclusion on the results of document-based GLP/GCP inspections and data integrity assessment

A document-based inspection and data integrity assessment were conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application. As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

2. PMDA's conclusion on the results of GCP on-site inspection

GCP on-site inspection took place in accordance with the provisions of the Pharmaceutical

Affairs Act for the data submitted in the new drug application (5.3.5.1-3, 5.3.5.1-4, 5.3.5.2-1). As a result, PMDA concluded that there should be no problem with conducting a regulatory review based on the submitted product application documents.

IV. Overall Evaluation

Based on the submitted data, PMDA concludes that the efficacy of the proposed product in patients with functional dyspepsia with chief gastrointestinal symptoms such as postprandial fullness, upper abdominal bloating, and early satiation has been demonstrated and its safety is acceptable in view of its observed benefits. PMDA considers that the proposed product may be approved if it can be concluded based on the comments raised by the Expert Discussion on the efficacy, safety, indications, dosage and administration, post-marketing investigations, etc., that there are no particular problems.

Review Report (2)

January 25, 2013

I. Product Submitted for Registration

[Brand name]	Acofide Tablets 100 mg
[Non-proprietary name]	Acotiamide Hydrochloride Hydrate
[Applicant]	Zeria Pharmaceutical Co., Ltd.
[Date of application]	September 29, 2010

II. Content of the Review

The Expert Discussion and subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined below. The expert advisors for the Expert Discussion were nominated based on their declarations, etc., concerning the product submitted for registration, in accordance with the provisions of the "Rules for Convening Expert Discussions, etc., by Pharmaceuticals and Medical Devices Agency" (PMDA administrative Rule No. 8/2008 dated December 25, 2008).

(1) Efficacy

PMDA considers as follows:

The phase III study showed a statistically significant difference between the 300 mg/day group and the placebo group in the "elimination rate of 3 symptoms at the last evaluation time point in the treatment period," one of the primary efficacy endpoints, which has demonstrated that acotiamide is effective in eliminating the 3 symptoms compared with the placebo. However, since the between-group difference in the extent of the efficacy with the placebo group is not sufficiently large, it is difficult to clearly explain the clinical significance of acotiamide from the aspect of the elimination of 3 symptoms. On the other hand, since FD is a disease associated only with subjective symptoms and the goal of the drug therapy is for patients to feel the improvement of symptoms, it is of clinical significance that a statistically significant difference was observed between the 300 mg/day group and the placebo group in the "improvement rate of subject's general impression at the last evaluation time point in the treatment period," the other primary endpoint, with a certain degree of between-group difference. On the basis of the overall evaluation of the two primary endpoints, PMDA concluded that the efficacy of acotiamide has been demonstrated.

The above conclusion of PMDA was supported by the expert advisors, together with the following comments raised from the expert advisors.

- The method for evaluating the efficacy of drugs for FD has hardly been established. In clinical practice, it is often the case that treatment efficacy is judged based on the subjective symptoms of patients and whether or not to continue treatment is determined based on this judgment. Therefore, it is important that, in the Phase III study, "improvement rate of subject's general impression at the last evaluation time point in the treatment period" was used as one of the primary endpoints. Also, it is of clinical significance that "elimination rate of 3 symptoms at the last evaluation time point in the treatment period," an index that allows more objective evaluation, was used as the other primary endpoint.

- Although the between-group difference in "elimination rate of 3 symptoms at the last evaluation time point in the treatment period" was as small as 6.3%, the fact that a statistically significant difference in the elimination rate of 3 symptoms observed in the acotiamide group

relative to the placebo group was appreciated, since it is difficult to achieve complete elimination of symptoms in FD patients. In addition, coupled with the results of "improvement rate of subject's general impression at the last evaluation time point in the treatment period," acotiamide has demonstrated a certain level of clinically significant efficacy in the treatment of FD.

(2) Safety

In the clinical studies involving FD patients, the incidence of adverse events was comparable between the acotiamide group and the placebo group, and there were only a few severe events. In the long-term treatment study, there were no adverse events that occurred more frequently in the long-term treatment study, although the number of patients who continued to receive acotiamide was limited. Based on the clinical study data submitted, PMDA concluded that the safety of acotiamide is acceptable.

The above conclusion of PMDA was supported by the expert advisors.

(3) Indications

PMDA considered as follows:

The Phase III study was conducted in patients with postprandial distress syndrome (PDS) characterized by postprandial fullness, upper abdominal bloating, and/or early satiation. Therefore, it should be clearly specified in "Indications" that acotiamide is useful for symptoms of PDS among those of functional dyspepsia. In contrast, efficacy has not been confirmed in patients with epigastric pain syndrome (EPS) characterized by epigastric pain and/or burning sensation. This information should also be provided in the package insert.

Regarding the duration of symptoms, the Rome III criteria requires that symptoms have been lasting for the last 3 months or longer with the onset at least 6 months prior to diagnosis. The clinical studies on acotiamide were conducted using this requirement as an inclusion criterion. On the other hand, in Japan, compared with the situation in other countries, it is easier for patients to receive care, including endoscopy, at medical institutions. Therefore, patients are more likely to visit medical institutions at a relatively early stage after the onset of symptoms, and it is unrealistic to strictly follow the Rome III criteria in administering acotiamide in clinical practice. Instead of specifying the exact duration of symptoms in the package insert, specific information obtained from patients in the Phase III study should be provided in the "Clinical Studies" section in the package insert.

Also, the diagnosis of FD should be made only after ruling out organic diseases. For this reason, it is necessary to raise cautions so that organic diseases should be ruled out appropriately by upper gastrointestinal endoscopy or any other appropriate means, together with thorough interview and examination before administering acotiamide.

The above conclusions of PMDA were supported by the expert advisors, together with the following comments raised from the expert advisors.

- Since the Phase III study was conducted in FD patients mainly with PDS symptoms, it is appropriate to clearly specify in the "Indications" that acotiamide is useful for PDS symptoms and to provide information in the package insert, on the lack of evidence for the efficacy in FD patients mainly with EPS symptoms.

- Although the Rome III criteria regarding the duration of symptoms is useful as inclusion criteria for clinical studies, they are not necessarily consistent with the circumstances in medical practice in Japan where it is easier for patients to receive care, including endoscopy, at medical institutions compared with situations in other countries. Therefore, PMDA's conclusion that the exact duration of symptoms need not be specified in the

package insert is agreeable. Also, it is important to provide information obtained from patients investigated in the Phase III study in the "Clinical Studies" section of the package insert.

- As is the case with non-erosive reflux disease, a disease similar to FD in not being accompanied by organic disease, caution should be raised to rule out organic diseases by upper gastrointestinal endoscopy or any other appropriate means.

- Given the mechanism of action of acotiamide, a temporary effect may be obtained even if organic disease is present. Therefore, it is important to rule out gastric cancer, etc., by upper gastrointestinal endoscopy or any other appropriate means.

On the basis of the above, PMDA asked the applicant to provide the following descriptions in "Indications" and "Precautions for Indications," to which the applicant responded appropriately, and PMDA accepted the response.

[Indications]
Postprandial fullness, upper abdominal bloating, and early satiation in patients with functional dyspepsia

[Precautions for Indications]
- Efficacy for epigastric pain and burning sensation in functional dyspepsia has not been confirmed.

- Organic diseases including malignant diseases such as gastric cancer should be excluded by upper gastrointestinal endoscopy or any other appropriate means.

Since physicians other than gastroenterologists may occasionally provide medical care to FD patients, it is necessary, for proper use of acotiamide, to provide appropriate information for the diagnosis and treatment of FD (e.g., definition of FD, diagnostic method including the method for ruling out organic diseases, treatment options other than acotiamide) to physicians including nonspecialists, using materials for physicians, etc. Furthermore, it is also necessary to promptly provide updated information including the guideline for diagnosis and treatment which is purported to be currently in preparation, in cooperation with relevant academic societies, etc.

The applicant agreed to comply with these instructions, and PMDA accepted the response.

(4) Dosage and Administration
PMDA considers as follows:
Regarding the dosage regimen of acotiamide, it is understandable that the daily dose of acotiamide was set at 300 mg in the Phase III study. Since the results showed that acotiamide was effective and had an acceptable safety profile, it is acceptable to set the daily dose at 300 mg. Also, it is understandable to recommend preprandial administration since PDS symptoms are considered to be related to food consumption.

In the long-term treatment study which set criteria for treatment suspension or discontinuation due to elimination of symptoms and for discontinuation because of no response (Table 44), of 405 evaluable patients, only 5 continued treatment for 48 weeks, suggesting that only a very limited number of patients will need long-term treatment with acotiamide. Therefore, caution should be raised not to continue treatment with acotiamide without careful consideration if symptoms remain improved over several weeks or if symptoms do not improve after treatment of approximately 1 month.

Furthermore, in the long-term treatment study, 1 patient died of pancreatic carcinoma (The patient had been suffering from FD for more than 1 year and upper gastrointestinal endoscopy detected no abnormality before acotiamide administration. On Day 208 after acotiamide administration was started, the patient was diagnosed with pancreatic carcinoma). It should be borne in mind that upper gastrointestinal endoscopy alone is difficult to make an accurate diagnosis, as was the case with this patient. Therefore, as explained by the applicant, it should be cautioned that patients should be monitored for their conditions even after the start of drug therapy, and additional tests such as ultrasonography should be performed as necessary if symptoms do not improve after a certain period of treatment.

The above conclusions of PMDA were supported by the expert advisors, together with the following comments raised from the expert advisors.

- The difference in the extent of efficacy between the placebo group and the acotiamide remained unchanged in the post-treatment period in the Phase III study (Figures 3 and 4). In the long-term treatment study, symptoms improved resulting in treatment suspension in 75.1% (304 of 405 patients), and the improvement continued over approximately 3 months in nearly half of the patients, resulting in study discontinuation. Theses findings provide important information for physicians and patients, many of whom may be anxious about discontinuing the treatment. It is therefore useful to provide such information in the "Clinical Studies" section in the package insert.

- In the results of the long-term treatment study (Figure 1), subjects who discontinued acotiamide because of symptom improvement or no response were excluded from the evaluation at each time point, while those who resumed taking acotiamide because of relapse after treatment suspension were included in the evaluation. These should be clearly indicated to avoid misinterpretation.

On the basis of the above, PMDA instructed the applicant to provide the following descriptions in "Dosage and Administration" and "Precautions for Dosage and Administration," to which the applicant responded appropriately, and PMDA accepted the response.

[Dosage and Administration]
The usual adult dosage is 100 mg of acotiamide hydrochloride hydrate administered orally three times daily before a meal.

[Precautions for Dosage and Administration]

- If symptoms do not improve after 1 month of treatment with acotiamide, consideration should be given to treatment discontinuation.

- If symptoms persist, the possibility of organic disease should be taken into account and consideration should be given to performing other tests in addition to upper gastrointestinal endoscopy, as necessary.

- When symptoms have remained improved over a sufficiently long period of time, consideration should be given to treatment discontinuation. Acotiamide should not be administered without careful consideration for a long period of time [see "Clinical Studies"].

Since physicians other than gastroenterologists may occasionally provide medical care to FD patients, it is important, for proper use of acotiamide, to familiarize physicians with the related precautions for use, etc., using materials for physicians, etc.

In actual clinical practice, acotiamide may be administered over a long period of time for more

than several months, depending on patient conditions. Since information on the long-term treatment of acotiamide is very limited, PMDA considers it necessary to collect safety information on long-term use of acotiamide, including treatment suspension and resumption, via post-marketing surveillance and, if it is found that there are a considerable number of patients receiving a long-term treatment, to collect and review further information on the safety of long-term treatment, as necessary.

The applicant agreed to address the above instructions, and PMDA accepted the response of the applicant.

(5) Post-marketing surveillance, etc.

PMDA considered as follows:

The clinical studies alone did not provide sufficient information on the safety in the elderly patients, etc., effect of *H. Pylori* (HP) infection, events induced by cholinergic action, safety of concomitant use with a cholinergic agent or an anti-cholinergic agent. The above information should be collected via post-marketing surveillance.

The above conclusion of PMDA was supported by the expert advisors, together with the following comments raised from the expert advisors.

- In the Phase III study, patients with organic diseases such as gastric erosions and esophageal hiatus hernia were excluded. This is consistent with the Rome III criteria which defines FD as symptoms with "no evidence of structural disease that is likely to explain the symptoms by upper endoscopy, etc." However, in clinical practice, symptoms may be often considered unrelated to these diseases, if present, and diagnosed with FD. To avoid such a misinterpretation, it is recommended that measures be taken to allow endoscopy findings to be described in the report form of post-marketing surveillance.

- It is recommended that accurate information be collected on acid secretion inhibitors which are likely to be concomitantly administered with acotiamide.

- There is no sufficient information currently available on the effect of HP infection in FD patients. Therefore, information should be collected on the effect of HP infection on the efficacy and safety of acotiamide via post-marketing surveillance.

- It should be thoroughly examined in advance what types of analysis can be performed based on the survey content and the number of the patients surveyed via post-marketing surveillance.

On the basis of the above, PMDA instructed the applicant to prepare the post-marketing surveillance plan. In response, the applicant submitted the outline of the post-marketing surveillance plan (draft) as the content of the use-results survey plan, as shown in Table 54, which PMDA accepted.

Table 54. Outline of use-results survey (draft)

Objectives	To investigate (i) unexpected adverse drug reactions, (ii) occurrence of adverse drug reactions in routine use, and (iii) factors affecting the safety and efficacy
Survey method	Central registration system
Patients surveyed	Patients for whom acotiamide is indicated
Survey duration	5 years, with 8-week survey period in each patient
Planned number of patients	3000
Main survey items	- Patient background characteristics (age, sex, medical history, complications, hepatic function, renal function, pregnancy, visit category, history of allergy, smoking habit, disease duration, whether endoscopy was performed or not, chief complaint, etc.) - Use status of acotiamide - Concomitant drugs or therapies - Presence or absence of *H. Pylori* infection and/or eradication - Adverse events that occurred during or after treatment with acotiamide - Overall evaluation of the efficacy of acotiamide (overall improvement, 3 major symptoms [postprandial fullness, upper abdominal bloating, early satiation], other subjective symptoms, etc.)

III. Overall Evaluation

As a result of the above review, PMDA concludes that the product may be approved after modifying the indications and the dosage and administration shown below. Since the product contains a new active ingredient, the re-examination period is 8 years. Neither the drug substance nor the drug product is classified as a poisonous drug or a powerful drug, and the product is not classified as a biological product or a specified biological product.

[Indications] Postprandial fullness, upper abdominal bloating, and early satiation in patients with functional dyspepsia

[Dosage and administration] The usual adult dosage is 100 mg of acotiamide hydrochloride hydrate administered orally three times daily before a meal.