Report on the Deliberation Results

June 22, 2015

Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau

Ministry of Health, Labour and Welfare

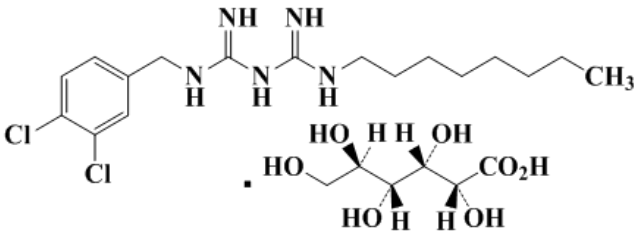
[Brand name]	Olanedine Antiseptic Solution 1.5%; Olanedine Solution 1.5% Antiseptic Applicator 10 mL; Olanedine Solution 1.5% Antiseptic Applicator 25 mL
[Non-proprietary name]	Olanexidine Gluconate (JAN*)
[Applicant]	Otsuka Pharmaceutical Factory, Inc.
[Date of application]	May 20, 2014
[Review results]	In the meeting held on June 17, 2015, the Second Committee on New Drugs concluded that the products may be approved and that its result should be presented to the Pharmaceutical Affairs Department of the Pharmaceutical Affairs and Food Sanitation Council. The re-examination period is 8 years. Neither the drug substance nor the drug product is classified as a poisonous drug, powerful drug, biological product, or specified biological product.
[Conditions for approval]	The applicant is required to develop and appropriately implement a risk management plan.

**Japanese Accepted Name (modified INN)*

Review Report

April 27, 2015
The Pharmaceuticals and Medical Devices Agency

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

[Brand name]	(1) Olanedine Antiseptic Solution 1.5% (2) Olanedine Solution 1.5% Antiseptic Applicator 10 mL; Olanedine Solution 1.5% Antiseptic Applicator 25 mL
[Non-proprietary name]	Olanexidine Gluconate
[Applicant]	Otsuka Pharmaceutical Factory, Inc.
[Date of application]	May 20, 2014
[Dosage form/Strength]	(1) A topical solution containing 3.0 g of Olanexidine Gluconate in one 200 mL bottle (2) A topical solution containing 0.15 g of Olanexidine Gluconate in one 10 mL applicator A topical solution containing 0.375 g of Olanexidine Gluconate in one 25 mL applicator
[Application classification]	Prescription drug (1) Drug with a new active ingredient
[Chemical structure]	 Molecular formula: $C_{17}H_{27}Cl_2N_5 \cdot C_6H_{12}O_7$ Molecular weight: 568.49 Chemical name:1-(3,4-Dichlorobenzyl)-5-octylbiguanide mono-D-gluconate
[Items warranting special mention]	None
[Reviewing office]	Office of New Drug IV

Review results

April 27, 2014

[Brand name] (1) Olanedine Antiseptic Solution 1.5%
(2) Olanedine Solution 1.5% Antiseptic Applicator 10 mL;
Olanedine Solution 1.5% Antiseptic Applicator 25 mL

[Non-proprietary name] Olanexidine Gluconate

[Applicant] Otsuka Pharmaceutical Factory, Inc.

[Date of application] May 20, 2014

[Review results]
Based on the submitted data, the Pharmaceuticals and Medical Devices Agency (PMDA) has concluded that the efficacy of the product for skin preparation at the surgical site has been demonstrated, and its safety is acceptable in view of observed benefits.

As a result of its regulatory review, PMDA concluded that these products may be approved for the following indication and dosage and administration, with the following conditions for approval.

[Indication] Preoperative skin preparation at the surgical site

[Dosage and administration] An appropriate amount of Olanedine is applied to the skin.

[Conditions for approval] The applicant is required to develop and appropriately implement a risk management plan.

Review Report (1)

March 9, 2015

I. Product Submitted for Registration

[Brand name] (1) Olanedine Antiseptic Solution 1.5%
(2) Olanedine Solution 1.5% Antiseptic Applicator 10 mL;
Olanedine Solution 1.5% Antiseptic Applicator 25 mL

[Non-proprietary name] Olanexidine Gluconate

[Applicant] Otsuka Pharmaceutical Factory, Inc.

[Date of application] May 20, 2014

[Dosage form/Strength] (1) A topical solution containing 3.0 g of Olanexidine Gluconate in one 200 mL bottle
(2) A topical solution containing 0.15 g of Olanexidine Gluconate in one 10 mL applicator
A topical solution containing 0.375 g of Olanexidine Gluconate in one 25 mL applicator

[Proposed indication] Preoperative skin preparation at the surgical site

[Proposed dosage and administration]
Olanedine is applied to the skin.

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

The submitted data and the review thereof by the Pharmaceuticals and Medical Devices Agency (PMDA) are summarized below.

1. Origin or history of discovery, use in foreign countries, and other information

Olanexidine, an antiseptic agent created by Otsuka Pharmaceutical Co., Ltd., is a chemical compound with a biguanide structure similar to chlorhexidine, and exerts a bactericidal effect on many species of Gram-positive and Gram-negative bacteria. Olanexidine hydrochloride hydrate, which was available in the early stage of development, was not sufficiently soluble at high concentrations. The applicant found that olanexidine gluconate is more soluble than olanexidine hydrochloride, and developed the former as an antiseptic agent for the skin.

Outside Japan, Otsuka America Pharmaceutical, Inc. was developing olanexidine gluconate topical solutions. However, in the United States, vacuolization of hepatocytes was observed in a non-clinical repeated-dose dermal toxicity study in rabbits. In addition, an increase in white blood cell count and an increase in the production of myeloid and granulocytic cells in the bone marrow were observed in repeated-dose subcutaneous toxicity studies in rats and dogs. In a clinical study in the United States (Study 168-06-220-11),

abnormal laboratory value in hepatic function and creatine kinase increased were reported (a causal relationship between the events and olanexidine gluconate was unknown in all subjects). On the basis of these findings suggesting possible hepatotoxicity and bone marrow toxicity associated with olanexidine, the U.S. Food and Drug Administration (FDA) placed a full clinical hold on the clinical development of olanexidine gluconate in the United States on [redacted], which was after the completion of phase I clinical studies in Japan. Subsequently, the FDA reviewed the results of additional toxicity studies (4-week dermal toxicity study in rabbits and minpigs) that were submitted on [redacted], and concluded that there are no safety concerns. The FDA changed the full clinical hold to a partial clinical hold and requested Otsuka America Pharmaceutical, Inc. to conduct a phase I clinical study to evaluate a single application of olanexidine gluconate. [redacted]

In Japan, the applicant concluded that clinical development of olanexidine gluconate solutions is feasible on the basis of the results of repeated-dose toxicity studies in rats and dogs, additional toxicity studies, and a phase I clinical study in Japan (Study 131-102). The applicant concluded that the efficacy and safety of an olanexidine gluconate 1.5% topical solution were demonstrated in Japanese clinical studies on the basis of the results of non-clinical studies and phase I studies (Studies 131-102 and 131-104), a phase II study (Study 131-201), a phase I/II study (Study 131-202), and phase III studies (Studies 131-301 and 131-302) in Japan. The applicant thus submitted a new drug application for olanexidine gluconate.

As of January 2015, no countries have approved any drug products containing olanexidine gluconate as an active ingredient.

2. Data relating to quality

2.A Summary of the submitted data

2.A.(1) Drug substance ([redacted])

[redacted]

[redacted]

2.A.(1).1) Characterization

[redacted]

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

See attachment.

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

[redacted]

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

Table 1 summarizes the results of stability tests conducted for the drug substance. The photostability study showed the drug substance to be photostable.

Table 1. Stability studies of drug substance

Study	Primary batches	Temperature	Humidity	Storage package	Storage period
Long-term	3 pilot batches	25°C	60% RH	[redacted]	12 months
Accelerated	3 pilot batches	40°C	75% RH		6 months

[redacted] The long-term testing will be continued for [redacted] months.

2.A.(2) Drug Product

2.A.(2).1) Description and composition of the drug product and formulation development

[redacted]

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

[redacted]

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

[redacted]

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

Table 2 summarizes the results of stability studies conducted for the drug product. The photostability studies showed the drug product to be photostable.

Table 2. Stability tests of drug product

Drug product	Study	Primary batches	Temperature	Humidity	Storage period	Storage package
Solution in bottle	Long-term	3 pilot batches	25°C	60% RH	36 months	[redacted]
	Accelerated	3 pilot batches	40°C	25% RH	6 months	
Solution in 10 mL applicator	Long-term	3 pilot batches	25°C	60% RH	24 months	[redacted]
	Accelerated	3 pilot batches	40°C	25% RH	6 months	
Solution in 25 mL applicator	Long-term	3 pilot batches	25°C	60% RH	24 months	[redacted]
	Accelerated	3 pilot batches	40°C	25% RH	6 months	

[redacted] The long-term testing of drug product solutions in the applicators will be continued for 36 months.

2.B. Outline of the review by PMDA

Based on the submitted data, PMDA concluded that the quality of the drug substance and drug product is appropriately controlled.

3. Non-clinical data

3.(i) Summary of pharmacology studies

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

Primary pharmacodynamics studies investigated the mechanism of action, *in vitro* bactericidal and antiviral effects, and *in vivo* bactericidal effects of olanexidine gluconate, as well as the possible development of resistant mutations against olanexidine gluconate. Secondary pharmacodynamic studies investigated cytotoxicity and the effect on wound healing of olanexidine gluconate. Safety pharmacology studies investigated the effects of olanexidine gluconate on the central nervous system, respiratory system, and cardiovascular system. Olanexidine hydrochloride hydrate (olanexidine hydrochloride) was used in the safety pharmacology studies. The applicant explained that the effects of olanexidine gluconate can be evaluated based on the results of safety pharmacology studies of olanexidine hydrochloride, since the results of a 4-week repeated subcutaneous toxicity study of olanexidine gluconate 6.95% solution (4.2.3.7.4-1) indicate that olanexidine gluconate and olanexidine hydrochloride are similar in terms of toxicity and pharmacokinetic profile. Unless otherwise specified, olanexidine hydrochloride doses are expressed as equivalent doses of olanexidine gluconate calculated based on the strength of olanexidine.

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

3.(i).A.(1).1) Mechanisms of action

(a) Binding affinity to lipopolysaccharides and lipoteichoic acid (4.2.1.1-1)

The BODIPY TR-cadaverine displacement assay,[1] an assay based on fluorescence intensity measurement, was conducted to determine the binding affinity of olanexidine gluconate to lipopolysaccharides and their hydrophobic component called lipid A on the outer membrane of Gram-negative bacteria as well as to lipoteichoic acid found in the cell wall of Gram-positive bacteria. The olanexidine concentration that showed 50% loss of fluorescence intensity was 20, 15, and 38 µmol/L (11, 8.7, and 21 µg/mL) for lipopolysaccharides, Lipid A, and lipoteichoic acid, respectively. The corresponding figures of chlorhexidine gluconate (CHG) were 160, 27, and 160 µmol/L (140, 25, and 140 µg/mL), respectively.

(b) Membrane disrupting effect (4.2.1.1-2)

The membrane disrupting effect of olanexidine gluconate was investigated using calcein-encapsulated large unilamellar vesicles (LUVs), a fluorescent probe. The results were expressed as percent leakage of calcein.[2] In an experiment using phosphatidyl ethanolamine-containing LUVs that were created as a model of bacterial membrane, the percentage leakage of calcein after 10-minute treatment with olanexidine gluconate 16 µmol/L, CHG 16 µmol/L, and vehicle were 96.1, 0.329, and -0.731, respectively. In an experiment using LUVs

[1] Wood SJ et al. *Comb Chem High Throughput Screen*. 2004;7:733-747.

[2] Percent leakage was calculated using the following formula:
% Leakage = $(F - F_0) \ / \ (F_{max} - F_0) \times 100$
Where F is the fluorescence intensity of the mixture of the test substance and LUV solution, F0 is the fluorescence intensity of the mixture of distilled water and LUV solution, and Fmax is the fluorescence intensity of the mixture of Triton X-100 (at a final concentration of 1%) and LUV solution.

containing phosphatidylcholine, a major phospholipid found in animal cell membrane, the percentage leakage of calcein after 10-minute treatment with olanexidine gluconate 16 μmol/L, CHG 16 μmol/L, and vehicle were 0.215, -0.266, and -0.908, respectively.

(c) Effects on bacterial cell membranes (4.2.1.1-3, 4.2.1.1-4)

Escherichia coli (*E. coli*) ML-35p was used to investigate the effect of olanexidine gluconate on the membrane permeability of the strain.[3] Olanexidine gluconate at ≥2.5 μg/mL and CHG at ≥2.5 μg/mL increased the permeability of the outer membrane of E. coli ML-35p, and Olanexidine gluconate at ≥5.0 μg/mL and CHG at ≥2.5 μg/mL increased the permeability of the inner membrane of the strain. The minimum inhibitory concentrations (MICs) of olanexidine gluconate and CHG against E. coli ML-35p were 4.0 μg/mL and 1.3 μg/mL, respectively.

The membrane potential-sensitive fluorescent dye DiSC3 (5) (3,3'-Dipropylthiadicarbocyanine iodide) was used to investigate the effect of olanexidine gluconate on the membrane of *Staphylococcus aureus* (*S. aureus*) ATCC29213. Olanexidine gluconate at ≥0.25 μg/mL and CHG at ≥5.0 μg/mL showed increased fluorescence intensity associated with a change in membrane potential. The applicant explained that olanexidine gluconate and CHG have bacterial cell membrane-disrupting effect. The MICs of olanexidine gluconate and CHG against *S. aureus* ATCC29213 were 0.63 μg/mL and 1.3 μg/mL, respectively.

(d) Protein denaturation effect (4.2.1.1-5)

A hemoglobin denaturation assay[4] was performed to investigate the protein denaturation effect of olanexidine gluconate, CHG, and vehicle. Hexadecylpyridinium chloride monohydrate was used as the positive control.[5] The protein denaturation effect was expressed as the hemoglobin denaturation ratio (HDR%).[6] The HDR% was 15% for olanexidine gluconate 0.016% (160 μg/mL), -1.2% for CHG 0.016% (160 μg/mL), 2.5% for vehicle, and 39% for hexadecylpyridinium chloride monohydrate 0.016% (160 μg/mL).

3.(i).A.(1).2) *In vitro* bactericidal effect and antiviral effect

(a) Bactericidal activity against standard bacterial strains (4.2.1.1-6 to 4.2.1.1-9)

A broth microdilution method was used to determine bactericidal activity of olanexidine gluconate and control agents against standard strains, namely Gram-positive cocci, Gram-positive rods, and Gram-negative bacteria. Tables 3, 4, and 5 summarize the minimal bactericidal concentrations (MBC) of the agents tested.

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[3] Change in the outer membrane permeability of E. coli was detected with the cleavage of nitrocefin, a substrate of beta -lactamases. Change in the inner membrane permeability of E. coli was detected with the cleavage of o-nitrophenil-beta-D-galactopyranoside, a substrate of beta-galactosidases.

[4] A method to indirectly measure the structural change of a region located near the heme molecule of hemoglobin.

[5] Hayashi T et al. *Toxicol In Vitro*. 1994;8:215-220, Hayashi T et al. *AATEX*. 1993;2:25-31.

[6] Percent HDR was calculated using the following formula:
HDR% = 100 - (A(TH) - A(TB)) / (A(WH) - A(WB)) × 100
Where A(TH) is the absorbance of the mixture of a test substance or positive control and hemoglobin solution, A(TB) is the absorbance of the mixture or a test substance or positive control and buffer solution, A(WH) is the absorbance of distilled water mixed with the hemoglobin solution, and A(WB) is the absorbance of distilled water mixed with the buffer solution.

Table 3. Bactericidal activity against standard strains of Gram-positive cocci

Bacterial species (number of strains)	Range of MBC (μg/mL)[a)]					
	Olanexidine gluconate		CHG		PVI	
	Treatment time		Treatment time		Treatment time	
	30 sec	3 min	30 sec	3 min	30 sec	3 min
Kocuria varians (1)	54.3	13.6	> 312.5	39.1	390.6	195.3
Kytococcus sedentarius (1)	434.4	27.1	39.1	9.8	781.3	390.6
Micrococcus luteus (1)	54.3	13.6	> 312.5	312.5	781.3	195.3
S. aureus (3)	434.4-868.8	108.6-434.4	> 1250	> 1250	390.6-781.3	195.3-390.6
Methicillin-resistant *S. aureus* (MRSA) (2)	434.4, 1737.5	108.6, 434.4	> 1250	156.3, > 1250	390.6, 781.3	195.3, 390.6
Staphylococcus capitis (1)	54.3	≤ 6.8	> 39.1	> 39.1	≤ 48.8	≤ 48.8
Staphylococcus epidermidis (*S. epidermidis*) (1)	217.3	54.3	78.1	19.5	195.3	97.7
Staphylococcus haemolyticus (1)	27.1	13.6	1250	19.5	390.6	390.6
Staphylococcus hominis (1)	217.3	27.1	> 312.5	> 312.5	390.6	390.6
Staphylococcus saprophyticus (1)	13.6	≤ 6.8	39.1	19.5	195.3	195.3
Staphylococcus sciuri (1)	27.1	≤ 6.8	> 1250	78.1	390.6	≤ 48.8
Staphylococcus simulans (1)	27.1	13.6	> 1250	312.5	390.6	97.7
Staphylococcus warneri (1)	217.3	13.6	> 312.5	> 312.5	781.3	≤ 48.8
Staphylococcus xylosus (1)	217.3	≤ 6.8	2500	78.1	390.6	97.7
Streptococcus agalactiae (1)	54.3	13.6	> 625	> 625	781.3	390.6
Streptococcus bovis (1)	108.6	≤ 6.8	> 1250	> 1250	781.3	195.3
Streptococcus pneumoniae (1)	27.1	13.6	> 2500	312.5	390.6	97.7
Streptococcus pyogenes (1)	27.1	27.1	> 156.3	> 156.3	390.6	97.7
Enterococcus avium (4)	54.3-217.3	13.6-54.3	> 1250	9.8-> 1250	390.6-781.3	97.7-390.6
Enterococcus casseliflavus (4)	27.1-217.3	≤ 6.8-13.6	> 2500	> 2500	≥ 50,000	195.3-390.6
Enterococcus cecorum (1)	108.6	13.6	> 1250	> 1250	781.3	195.3
Enterococcus dispar (1)	13.6	≤ 6.8	> 1250	> 1250	781.3	390.6
Enterococcus faecalis (*E. faecalis*) (6)	27.1-108.6	≤ 6.8-13.6	> 5000	≥ 5000	≥ 50,000	≤ 48.8-1562.5
E. faecalis (VRE) (3)	54.3	≤ 6.8-13.6	> 5000	> 5000	≥ 50,000	390.6-781.3
Enterococcus faecium (6)	13.6-217.3	≤ 6.8-27.1	> 1250	≥ 1250	195.3-> 50,000	≤ 48.8-1562.5
Enterococcus faecium (VRE) (2)	13.6, 27.1	13.6	≥ 5000	2500, 5000	781.3	97.7
Enterococcus flavescens (2)	13.6, 54.3	13.6	> 2500	> 2500	781.3, 50,000	390.6, 781.3
Enterococcus gallinarum (2)	108.6, 217.3	13.6, 27.1	> 5000	≥ 5000	≥ 50,000	390.6, 781.3
Enterococcus gilvus (1)	434.4	54.3	> 1250	> 1250	781.3	390.6
Enterococcus pallens (1)	434.4	54.3	> 625	> 625	781.3	781.3
Enterococcus raffinosus (1)	108.6	13.6	> 1250	> 1250	1562.5	781.3

PVI, povidone iodine; VRE, vancomycin-resistant *Enterococcus* species.

a) The range of MBCs against the testing strains is indicated. When 1 or 2 strains were used for a bacterial species, individual MBCs are indicated. When all strains of a particular species showed the same MBC, the MBC is indicated.

Table 4. Bactericidal activity against standard strains of Gram-positive rods

Bacterial species (1 strain each)	MBC (μg/mL)					
	Olanexidine gluconate		CHG		PVI	
	Treatment time		Treatment time		Treatment time	
	30 sec	3 min	30 sec	3 min	30 sec	3 min
Brevibacterium epidermidis	13.6	≤ 6.8	1250	19.5	781.3	97.7
Corynebacterium diphtheriae (*C. diphtheriae*)	108.6	13.6	> 312.5	> 312.5	781.3	390.6
Corynebacterium jeikeium	108.6	27.1	> 625	625	195.3	97.7
Corynebacterium minutissimum	54.3	13.6	> 625	625	97.7	97.7
Corynebacterium xerosis	108.6	≤ 6.8	> 156.3	78.1	390.6	97.7
Listeria monocytogenes	868.8	≤ 6.8	> 2500	> 2500	781.3	195.3
Propionibacterium acnes	1737.5	108.6	> 312.5	156.3	781.3	390.6
Propionibacterium avidum	27.1	13.6	625	78.1	781.3	781.3
Propionibacterium granulosum	≤ 6.8	≤ 6.8	19.5	9.8	781.3	781.3

Table 5. Bactericidal activity against standard strains of Gram-negative bacteria

Bacterial species [b]	MBC (μg/mL)					
	Olanexidine gluconate		CHG		PVI	
	Treatment time		Treatment time		Treatment time	
	30 sec	3 min	30 sec	3 min	30 sec	3 min
Achromobacter xylosoxidans	434.4	27.1	> 5000	2500	390.6	195.3
Acinetobacter baumannii (*A. baumannii*)	13.6	≤ 6.8	312.5	19.5	195.3	97.7
Acinetobacter calcoaceticus	54.3	13.6	1250	19.5	195.3	97.7
Acinetobacter species	27.1	≤ 6.8	156.3	19.5	390.6	≤ 48.8
Alcaligenes faecalis	868.8	54.3	5000	1250	3125	390.6
Bacteroides fragilis	54.3	13.6	312.5	156.3	781.3	781.3
Burkholderia cepacia (*B. cepacia*)	> 6950	434.4	> 5000	625	390.6	195.3
Citrobacter freundii	108.6	13.6	1250	39.1	781.3	390.6
Enterobacter aerogenes	54.3	≤ 6.8	625	19.5	390.6	195.3
Enterobacter cloacae	108.6	27.1	1250	78.1	781.3	390.6
E. coli	54.3, 217.3	13.6, 27.1	39.1, 625	19.5, 39.1	390.6, 781.3	195.3
Klebsiella pneumoniae (*K. pneumoniae*)	54.3	13.6	2500	156.3	781.3	390.6
Legionella pneumophila	13.6	13.6	> 78.1	> 78.1	195.3	195.3
Moraxella catarrhalis	13.6	≤ 6.8	312.5	19.5	≤ 48.8	≤ 48.8
Morganella morganii	217.3	13.6	39.1	9.8	195.3	≤ 48.8
Pantoea agglomerans	217.3	27.1	312.5	39.1	390.6	195.3
Proteus hauseri	54.3	≤ 6.8	156.3	39.1	390.6	97.7
Proteus mirabilis	868.8	54.3	> 5000	39.1	390.6	97.7
Providencia rettgeri	54.3	13.6	5000	156.3	390.6	390.6
Providencia stuartii	868.8	54.3	> 5000	2500	781.3	390.6
Pseudomonas aeruginosa (*P. aeruginosa*)	54.3	13.6	39.1, > 5000	19.5, 78.1	390.6, 781.3	195.3, 390.6
Pseudomonas fluorescens	≤ 6.8	≤ 6.8	78.1	≤ 4.9	390.6	195.3
Pseudomonas putida	27.1	13.6	156.3	9.8	781.3	390.6
Pseudomonas stutzeri	13.6	13.6	156.3	9.8	195.3	97.7
Salmonella enteritidis	54.3	≤ 6.8	78.1	19.5	390.6	195.3
Salmonella paratyphi	27.1	13.6	156.3	39.1	390.6	195.3
Salmonella typhimurium	54.3	≤ 6.8	5000	156.3	781.3	390.6
Serratia liquefaciens	54.3	13.6	1250	39.1	781.3	781.3
Serratia marcescens (*S. marcescens*)	54.3	13.6	5000	39.1	390.6	195.3
Shigella flexneri	217.3	27.1	> 625	19.5	781.3	97.7
Shigella sonnei	54.3	≤ 6.8	1250	19.5	390.6	195.3
Stenotrophomonas maltophilia	108.6	13.6	5000	19.5	390.6	97.7
Yersinia enterocolitica	217.3	13.6	625	19.5	390.6	97.7

a) When 2 strains were used for a bacterial species, individual MBCs are indicated. When both strains showed the same MBC, the MBC is indicated.

b) Two strains were used for *B. cepacia*, *E. coli*, and *P. aeruginosa*, and 1 strain for other bacterial species.

(b) Bactericidal activity against standard fungal strains (4.2.1.1-7, 4.2.1.1-10)

A broth microdilution method was used to determine fungicidal activity of olanexidine gluconate and control agents against standard fungal strains. Table 6 summarizes the results.

Table 6. Fungicidal activity against standard fungal strains

Fungal species (1 strain each)	MBC (μg/mL)					
	Olanexidine gluconate		CHG		PVI	
	Treatment time		Treatment time		Treatment time	
	30 sec	3 min	30 sec	3 min	30 sec	3 min
Candida albicans	868.8	108.6	5000	156.3	781.3	781.3
Candida guilliermondii	868.8	27.1	156.3	≤ 4.9	781.3	781.3
Candida parapsilosis	> 6950	434.4	> 5000	> 5000	781.3	781.3
Candida tropicalis	868.8	54.3	156.3	78.1	1562.5	390.6
Cryptococcus neoformans	6950	217.3	5000	2500	781.3	781.3
Malassezia furfur	3475	434.4	5000	5000	6250	781.3
Aspergillus fumigatus	> 3475	217.3	> 2500	> 2500	1562.5	1562.5
Aspergillus niger	> 6950	> 6950	> 5000	> 5000	> 25,000	> 25,000
Epidermophyton floccosum	108.6	27.1	> 2500	78.1	195.3	97.7
Microsporum canis	> 6950	> 6950	> 5000	> 5000	3125	3125
Microsporum gypseum	> 3475	3475	> 5000	> 5000	1562.5	1562.5
Trichophyton mentagrophytes	> 3475	868.8	> 2500	> 2500	781.3	781.3
Trichophyton rubrum	3475	108.6	> 5000	625	390.6	390.6

(c) Bactericidal activity against standard strains of spore-forming bacteria (4.2.1.1-11)

A broth microdilution method was used to determine bactericidal activity of olanexidine gluconate and control agents against standard strains of spore-forming bacteria after treatment with each agent for 3, 30, and 60 minutes. The MBCs of olanexidine gluconate, CHG, and PVI against *Bacillus subtilis* were >3475 μg/mL, >625 μg/mL, and >50,000 μg/mL, respectively, irrespective of treatment time (3, 30, or 60 minutes). The corresponding figures against *Clostridium sporogenes* was >6950 μg/mL, >5000 μg/mL, and >50,000 μg/mL, respectively.

(d) Bactericidal activity against clinical isolates (4.2.1.1-12 to 4.2.1.1-14)

A broth microdilution method was used to determine bactericidal activity of olanexidine gluconate and control agents against clinical isolates of bacteria causing surgical wound infection.[7] Table 7 summarizes the results.

[7] Surgical Site Infection Division of the Japan Nosocomial Infections Surveillance. The Ministry of Health, Labor and Welfare. Nosocomial infection surveillance annual reports in 2011 and 2012.
http://www.nih-janis.jp/report/ssi.html (Accessed in February 2015)

Table 7. Bactericidal activity against clinical isolates

Bacterial species (number of strains)	Treatment time	Olanexidine gluconate	CHG	PVI
		Range of MBC (μg/mL)	Range of MBC (μg/mL)	Range of MBC (μg/mL)
MRSA (30)	30 sec	217.3->3475	2500->5000	781.3-1562.5
	1 min	217.3-868.8	2500->5000	781.3-1562.5
	3 min	54.3-217.3	2500->5000	195.3-781.3
E. faecalis (30)	30 sec	54.3-434.4	625->5000	781.3-50,000
	1 min	27.1-217.3	312.5->5000	390.6-3125
	3 min	13.6-108.6	156.3-5000	195.3-1562.5
Methicillin-sensitive *S. aureus* (20)	30 sec	217.3->3475	156.3->2500	390.6-1562.5
	1 min	217.3-1737.5	78.1->2500	195.3-781.3
	3 min	≤6.8-868.8	≤4.9-156.3	≤48.8-781.3
E. coli (20)	30 sec	54.3-217.3	19.5-625	195.3-781.3
	1 min	54.3-217.3	9.8-312.5	97.7-781.3
	3 min	27.1-108.6	≤4.9-78.1	≤48.8-390.6
Coagulase-negative *Staphylococcus* species(CNS) (20)	30 sec	217.3->868.8	156.3->1250	195.3-1562.5
	1 min	108.6-868.8	39.1->1250	97.7-1562.5
	3 min	13.6-108.6	19.5->625	≤48.8-781.3
Corynebacterium species (20)	30 sec	≤6.8-54.3	≤4.9->78.1	≤48.8-781.3
	1 min	≤6.8-27.1	≤4.9->78.1	≤48.8-390.6
	3 min	≤6.8-13.6	≤4.9-78.1	≤48.8-390.6
P. aeruginosa (20)	30 sec	27.1-868.8	39.1->5000	195.3-781.3
	1 min	13.6-217.3	39.1->5000	195.3-781.3
	3 min	≤6.8-54.3	19.5-312.5	195.3-781.3
K. pneumoniae (20)	30 sec	13.6-54.3	19.5-78.1	195.3-781.3
	1 min	≤6.8-27.1	19.5-78.1	97.7-390.6
	3 min	≤6.8-27.1	9.8-39.1	≤48.8-390.6
A. baumannii (20)	30 sec	13.6-108.6	39.1-156.3	≤48.8-390.6
	1 min	13.6-54.3	19.5-156.3	≤48.8-390.6
	3 min	13.6-27.1	9.8-78.1	≤48.8-390.6
S. marcescens (20)	30 sec	27.1-3475	78.1->5000	97.7-390.6
	1 min	13.6-434.4	39.1->5000	97.7-390.6
	3 min	13.6-217.3	19.5-78.1	97.7-390.6

(e) Antiviral activity (4.2.1.1-15)

The 50% tissue culture infective dose ($TCID_{50}$) of olanexidine gluconate and control agents against test strains was determined. Influenza A virus (H1N1) ATCC VR-1469, an enveloped virus, was treated with olanexidine gluconate 1.5% solution, CHG 0.5% solution, and PVI 10 % solution for 1 minute. The log reduction values (LRVs)[8] were 4.95[9] for olanexidine gluconate, 0.05 for CHG, and 4.95[9] for PVI. Feline calicivirus ATCC VR-782, a nonenveloped virus, was also treated with these solutions for 1 minute. The LRVs were 0.60 for olanexidine gluconate, 0.10 for CHG, and 5.10[9] for PVI.

(f) Resistance profile (4.2.1.1-16)

Bacterial resistance to olanexidine gluconate and CHG was studied by serial passage. Table 8 summarizes the results.

[8] The LRVs were calculated by subtracting the viral infectivity titer in the presence of a test agent (Log_{10} $TCID_{50}$/mL) from the viral infectivity titer in the presence of a negative control agent (Log_{10} $TCID_{50}$/mL). The Spearman-Kärber method was used to calculate viral infectivity titer. When the viral infectivity titer in the presence of a test agent was below the detection limit, the LRV was calculated by subtracting the detection limit value from the viral infectivity titer of the negative control agent.

[9] The viral infectivity titer was below the lower detection limit.

Table 8. Bacterial resistance to olanexidine gluconate and CHG

Bacterial species	Agents tested[a)]	MIC (μg/mL) at each passage														
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15[b)]
A. baumannii	Olanexidine gluconate	2	2	2	2	2	2	4	4	4	4	2	4	4	4	4
	CHG	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
B. cepacia	Olanexidine gluconate	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	CHG	4	4	4	4	4	4	4	4	4	8	8	16	8	8	8
E. faecalis	Olanexidine gluconate	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	CHG	2	4	4	4	4	4	4	4	4	4	4	4	4	4	4
E. coli	Olanexidine gluconate	4	4	4	4	4	4	4	4	4	4	4	8	8	8	8
	CHG	2	1	1	2	1	1	1	1	1	1	1	1	1	1	1
K. pneumoniae	Olanexidine gluconate	8	8	8	8	8	4	8	8	8	8	4	8	4	8	4
	CHG	32	64	64	64	64	64	64	64	64	128	128	128	128	128	64
P. aeruginosa	Olanexidine gluconate	8	8	16	16	16	16	16	32	64	64	64	64	64	64	64
	CHG	8	8	16	16	16	16	16	16	16	16	16	16	16	16	16
S. marcescens	Olanexidine gluconate	8	16	512	512	512	512	512	512	512	512	512	512	512	512	256
	CHG	32	64	64	64	64	64	64	128	256	256	256	512	512	512	64
S. aureus	Olanexidine gluconate	0.5	1	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1
	CHG	0.5	0.5	0.5	0.5	1	1	1	1	1	1	1	1	1	1	1
S. epidermidis	Olanexidine gluconate	1	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	CHG	0.5	0.5	1	0.5	1	1	0.5	1	0.5	1	1	1	1	1	1

a) The applicant explained that as olanexidine gluconate at ≥32 μg/mL and CHG at ≥128 μg/mL were not soluble, the measured MICs may be higher than the true MICs at these concentrations.
b) MIC was determined after 14 consecutive passages. MIC was determined again after an additional 2 passages in the absence of the test substances to determine the final MIC.

3.(i).A.(1).3) *In vivo* studies

(a) Bactericidal activity against a mouse skin infection model (4.2.1.1-17 to 4.2.1.1-25)

The bactericidal activity of olanexidine gluconate and control agents against selected bacterial species was evaluated using a mouse skin infection model. Table 9 summarizes the results.

Table 9. Bactericidal activity against selected bacterial species in a mouse skin infection model

Bacterial species	Treatment time	Control		Olanexidine gluconate 1.5%		CHG 0.5%		PVI 10%	
		Viable cell count	Mean bactericidal rate (%)	Viable cell count	Mean bactericidal rate (%)	Viable cell count	Mean bactericidal rate (%)	Viable cell count	Mean bactericidal rate (%)
S. aureus	30 sec	5.36	-	1.82	99.96	4.78	72.66	2.44	99.84
	3 min	5.39	-	0	> 99.99	3.45	97.76	0.26	> 99.99
S. epidermidis	30 sec	4.93	-	0.41	> 99.99	4.53	56.17	0.53	99.18
	3 min	4.92	-	0.19	> 99.99	3.28	97.30	0.00	> 99.99
A. baumannii	30 sec	5.35	-	0.10	> 99.99	0.39	> 99.99	0.27	> 99.99
	3 min	5.32	-	0.20	> 99.99	0.20	> 99.99	0	> 99.99
C. diphtheriae	30 sec	4.92	-	0.30	> 99.99	1.35	99.91	0.47	99.99
	3 min	4.90	-	0.17	> 99.99	0.39	99.99	0.10	> 99.99
MRSA	30 sec	5.53	-	0.64	> 99.99	3.88	96.24	2.10	99.84
	3 min	5.56	-	0.30	> 99.99	2.32	99.93	0.33	> 99.99
E. faecalis (VRE)	30 sec	5.57	-	0.30	> 99.99	4.45	91.33	4.59	89.08
	3 min	5.57	-	0.44	> 99.99	2.82	99.40	2.95	98.34
P. aeruginosa	30 sec	5.49	-	0.40	99.99	1.39	99.98	0.24	> 99.99
	3 min	5.50	-	0.20	> 99.99	0.39	> 99.99	0.14	> 99.99
S. marcescens	30 sec	5.66	-	1.85	99.32	2.12	99.94	0.39	> 99.99
	3 min	5.63	-	0.77	99.93	0.94	> 99.99	0.10	> 99.99
B. cepacia	30 sec	-	-	-	-	-	-	-	-
	3 min	4.52	-	1.58	99.78	4.15	26.78	1.25	99.93

Mean (n=7/species); Log_{10} CFU/3.5cm^2; The minus (-) symbol means "not measured."

(b) Bactericidal activity against normal and transient skin flora in monkeys (4.2.1.1-26)

Abdominal skin samples were collected from cynomolgus monkeys before and after application of olanexidine gluconate 1.5% solution, CHG 0.5% solution, PVI 10% solution, or physiological saline, to determine bactericidal activity of these substances against normal and temporal skin flora.[10] Bactericidal activity measured after 10-minute application were 3.41 for olanexidine gluconate, 3.24 for CHG, 3.71 for PVI, and 0.92 for physiological saline.

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

3.(i).A.(2).1) *In vitro* studies

Cytotoxicity studies using established human cell lines (4.2.1.2-1 to 4.2.1.2-5)

As olanexidine gluconate 1.5% solution is to be applied directly to surgical site for preoperative skin preparation, internal organs may be exposed to olanexidine gluconate. Thus, a study was conducted to investigate the effect (invasiveness) of olanexidine gluconate and CHG on organs and tissues directly exposed to the agents. Cytotoxicity of the test substances on human cell lines was expressed as the concentration resulting in 50% survival of cells (50% inhibitory concentration [IC_{50}]).[11] Table 10 summarizes the results. The range of IC_{50} values in each cell line did not differ substantially between olanexidine gluconate (0.00034% to 0.0013%; 6.0 to 22 μmol/L) and CHG (0.00034% to 0.0015%; 3.7 to 16 μmol/L). No specific cell line showed a significant difference in IC_{50} value between olanexidine gluconate and CHG. Accordingly, the applicant explained that olanexidine gluconate and CHG do not differ substantially in terms of cytotoxicity.

Table 10. Cytotoxicity (IC_{50}) against established human cell lines

Cell line (source)	Olanexidine gluconate		CHG	
	Concentration (%)	Concentration (μmol/L)	Concentration (%)	Concentration (μmol/L)
HCM cells (heart)	0.00037	6.4	0.0015	16
A549 cells (lung)	0.00071	12	0.00051	5.7
IMR-90 cells (lung)	0.00043	7.5	0.0014	16
HepG2 cells (liver)	0.0006	11	0.00061	6.8
HEK 293 cells (kidney)	0.00034	6	0.00034	3.7
MKN 45 cells (stomach)	0.00046	8.1	0.00061	6.8
PANC-1 cells (pancreas)	0.00038	6.8	0.00064	7.1
Caco-2 cells (colon)	0.0013	22	0.0012	13
HT-29 cells (colon)	0.00041	7.3	0.00053	5.9
RMS-YM cells (skeletal muscle)	0.00044	7.8	0.00059	6.5

3.(i).A.(2).2) *In vivo* studies

Effects on skin wound healing (4.2.1.2-6)

A mouse model of full-thickness skin defect was used to evaluate the effect of olanexidine gluconate on skin

[10] Viable cell count at each time point (Log_{10} CFU/cm^2) was subtracted from viable cell count at baseline (Log_{10} CFU/cm^2).

[11] The percentage of viable cells was determined after 48 hours of treatment for all cell lines other than HCM, for which viable cells were counted after 24 hours of treatment.

wound healing. The wound area rate[12] at 14 days after application was 15.3% for olanexidine gluconate 2% solution and 30.6% for physiological saline, but did not differ substantially at any other time points.[13] The number of days to wound healing and general condition and body weight of animals did not differ between the two solutions.

3.(i).A.(3) Safety pharmacology (4.2.1.3-1 to 4.2.1.3-3)

The effects of olanexidine hydrochloride on the central nervous system, the respiratory system, and the cardiovascular system were evaluated. Table 11 summarizes the results.

Table 11. Outline of safety pharmacology studies

Organ system	Test system	Route of administration	Dose	No. of animals	Findings
Central nervous system (FOB method)	SD rats	Subcutaneous	0.136, 1.36, 13.6 mg/kg	6 males	13.6 mg/kg: High body temperature, bulges in the back and axillary region, and pale brown pigmented hypertrophy and pale brown pigmented edematous hypertrophy in subcutaneous tissues of the administration site.
Respiratory system	SD rats	Subcutaneous	0.136, 1.36, 13.6 mg/kg	6 males	13.6 mg/kg: Increases in respiratory rate, tidal volume, and respiratory minute volume, and pale brown pigmented hypertrophy of subcutaneous tissues
Cardiovascular system (telemetry study)	Beagle dogs	Intravenous	0.0408, 0.408, 4.08 mg/kg	4 males	4.08 mg/kg: Decreases in heart rate, systolic blood pressure, diastolic blood pressure, and mean blood pressure

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

3.(i).B.(1) Mechanism of action and bactericidal activity of olanexidine gluconate

The applicant's explanation on the mechanism of action and bactericidal activity of olanexidine gluconate:

On the basis of the studies on binding affinity to lipopolysaccharides and lipoteichoic acid, membrane disrupting effect, and the effect on membrane permeability of E. coli and membrane potential of *S. aureus* (see "3.(i).A.(1).1) Mechanisms of action"), olanexidine gluconate is considered to exert a bactericidal activity by binding to bacterial membranes and disrupting the membrane structure to cause irreversible leakage of cytoplasmic components. High concentrations of olanexidine gluconate (≥160 μg/mL) exerts a protein denaturation effect (see "3.(i).A.(1).1) Mechanisms of action"), resulting in aggregation[14] and death of bacteria.

The purpose of skin preparation before surgery is to reduce the normal skin flora.[15] Primary normal skin flora

[12] The wound area rate was calculated using the following formula: Wound area rate (%) = (wound area at a specific time point / baseline wound area) × 100

[13] Animals were observed on the day of application and 3, 7, 10, 14, 17, 21, 24, and 28 days after the application.

[14] Sakagami Y et al. *J Pharm Pharmacol*. 1999;51:201-206.

[15] Sebben JE. *J Am Acad Dermatol*. 1983;9:759-765.

consists of 6 bacterial genera, namely Gram-positive cocci (*Staphylococcus* sp. and *Micrococcus* sp.), Gram-positive rods (*Corynebacterium* sp., *Propionibacterium* sp., and *Brevibacterium* sp.), and Gram-negative rods (*Acinetobacter* sp.).[16] Major causes of surgical wound infection include *S. aureus* (including methicillin-resistant S. aureus [MRSA]), coagulase-negative *Staphylococcus* sp, *Enterococcus* sp. (including vancomycin-resistant enterococci [VRE]), *E. coli*, and *P. aeruginosa*.[7] Three-minute treatment with olanexidine gluconate at <0.086% concentrations (< 868.8 μg/mL) exhibited bactericidal effects against Gram-positive bacteria, including the above species (not including spore-forming bacteria) and Gram-negative bacteria (see "3.(i).A.(1).2) *In vitro* bactericidal effect and antiviral effect"). These concentrations are lower than the concentration of olanexidine gluconate to be used in the clinical setting (1.5%; 15,000 μg/mL).

The applicant considered that the above findings demonstrated the bactericidal effect of olanexidine gluconate against common causative bacteria of surgical wound infections and normal skin flora.

PMDA's view:
Olanexidine gluconate is expected to exert bactericidal activity against various bacterial species, including common causative bacteria of surgical wound infection and normal skin flora. The clinical efficacy of olanexidine gluconate applied to the skin will be described in "4.(iii).B.(1) Efficacy."

3.(i).B.(2) Resistance to olanexidine gluconate

PMDA asked the applicant to provide the latest findings on the occurrence of antiseptic-resistant bacteria.

The applicant's explanation:
In Taiwan, a hospital where an antiseptic liquid containing CHG 4% has been used for ≥ 20 years experienced an increase in the percentage of MRSA strains with high MICs for CHG, from 1.7% in 1990 to approximately 50% in 1995 to 2005. The acquisition of the efflux-mediated antiseptic resistance gene qacA/B may have contributed to the development of resistance to CHG.[17] In the hospital, however, the MIC of CHG against MRSA strains ranged from 1 to 16 μg/mL in 2005; this suggests that antiseptic agents at clinical concentrations[18] are unlikely to induce the development of antiseptic-resistant strains that may affect the clinical efficacy of the agents.

PMDA's view on the development of olanexidine gluconate-resistant bacteria:
The use of CHG, a drug in the same class as olanexidine gluconate, has been reported to increase its MIC against MRSA. Further, the serial passage to assess the bacterial resistance profile against olanexidine

[16] Wilson M. Microbial inhabitants of humans. 2005;65-76, Miyaji Y. Naganuma M, Dermatology for researchers of cosmetics and drugs for external use. 2005;40-44.
[17] Wang JT et al. *J Antimicrob Chemother*. 2008;62:514-517.
[18] It is explained that CHG 0.1% to 0.5% aqueous solutions (1000 to 5000 μg/mL) are used for preoperative skin preparation at the surgical site.

gluconate has suggested increases in MICs against *P. aeruginosa* and *S. marcescens*, although the appropriateness of MIC measurement was questioned due to the low solubility of olanexidine gluconate at high concentrations (see "(f) Resistance profile (4.2.1.1-16)" under "3.(i).A.(1).2) *In vitro* bactericidal effect and antiviral effect"). Thus the applicant should continue collecting information on bacterial resistance to olanexidine gluconate in the post-marketing settings and appropriately provide healthcare professionals with new findings available.

3.(ii) Summary of pharmacokinetic studies

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

The applicant submitted data on the pharmacokinetics of olanexidine in rats, dogs, rabbits, and minipigs that received ^{14}C-labeled and unlabeled olanexidine gluconate and unlabeled olanexidine hydrochloride intravenously, subcutaneously, and epidermally. Unless otherwise specified, olanexidine hydrochloride doses are expressed as equivalent doses of olanexidine gluconate calculated based on the strength of olanexidine. The concentrations of olanexidine and its metabolites in biological samples were determined by high performance liquid chromatography (HPLC) with ultraviolet detection or liquid chromatography-tandem mass spectrometry.[19] Radioactivity concentrations in biological samples were determined by liquid scintillation counting technique or HPLC with continuous radioactivity detection. Radioactivity concentrations in whole-body sections were determined by radioluminography.

Unless otherwise specified, pharmacokinetic parameters are expressed as mean values.

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

3.(ii).A.(1).1) Single-dose studies (4.2.2.2-1 to 4.2.2.2-5)

Male rats (3/time point) received a single intravenous dose of olanexidine gluconate at 0.139, 0.417, and 1.39 mg/kg, and serum olanexidine concentrations were determined. Pharmacokinetic parameters of olanexidine after intravenous administration of 0.139, 0.417, and 1.39 mg/kg were as follows: The initial concentrations (C_0) were 43.5, 157, and 344 ng/mL, respectively. The areas under concentration-time curves from zero to infinity (AUC_{inf}) were 31.4, 112, and 286 ng·h/mL, respectively. Total body clearance was 2900, 2487, and 3255 mL/h/kg, respectively. The volume of distribution was 8746, 7296, and 11,845 mL/kg, respectively. At all doses tested, serum olanexidine concentration decreased in a biphasic manner. The elimination half-life ($t_{1/2}$) ranged between 0.23 and 0.26 hours during the period from 5 to 30 minutes postdose, and between 4.4 to 5.7 hours during the period from 4 to 24 hours postdose.

Rats (3/sex/time point) and 3 male dogs received a single subcutaneous injection of ^{14}C-labeled olanexidine

[19] The lower limits of quantification of olanexidine and its metabolites are as follows:
In rats, 0.05 ng/mL for olanexidine and 3, 4-dichlorobenzoic acid (DCBA); 0.005 ng/mL for DM-210 and DM-212.
In rabbits, 0.05 ng/mL for olanexidine; 0.005 ng/mL for DM-210 and DM-212.
In minipigs, 0.05 ng/mL for olanexidine, DM-210, and DM-211.

gluconate at 1.39 mg/kg, and serum and blood radioactivity concentrations were determined. Table 12 summarizes pharmacokinetic parameters of olanexidine.

Table 12. Pharmacokinetic parameters of olanexidine after a single subcutaneous injection of ^{14}C-labeled olanexidine

Animal species	No. of animals	C_{max} [a)]		T_{max} (h)		AUC_{inf} [b)]		$t_{1/2}$ (h)[c)]	
		Serum	Blood	Serum	Blood	Serum	Blood	Serum	Blood
Rats	3 males/time point	33.8	44.1	4	1	716	894	20 (6-72h)	22 (24-72h)
	3 females/time point	23.1	35.8	2	2	500	696	23 (6-72h)	21 (6-72h)
Dogs	3 males	96.9	83.2	8	7	13,900	11,000	71.4 (96-168h)	71.0 (96-168h)

Mean values; C_{max}, peak concentration; T_{max}, time to peak concentration
a) Serum concentrations are expressed in ng eq./mL, and blood concentrations in ng eq./g.
b) AUC in serum are expressed in ng eq.·h/mL, and AUC in blood in ng eq.·h/g.
c) Olanexidine concentrations in serum and blood decreased in a polyphasic manner. The elimination half-life was calculated over the time periods indicated in parentheses.

Rats (3/sex/time point) received a single subcutaneous injection of olanexidine hydrochloride at 1360 mg/kg. The peak serum olanexidine concentration (C_{max}) was 81.8 ng/mL, AUC from 0 to 72 hours was 3374 ng·h/mL, and T_{max} was 8 hours.

3.(ii).A.(1).2) Repeat dose studies (4.2.2.2-7, 4.2.3.2-7, 4.2.3.5.2-3, 4.2.3.7.4-1)

Male and female rats (9/sex/group) received repeated subcutaneous injections of olanexidine gluconate at 0.0278, 0.278, or 2.78 mg/kg/day, and olanexidine hydrochloride at 0.0272, 0.272, or 2.72 mg/kg/day for 4 weeks. Table 13 summarizes C_{max} and AUC from 0 to 24 hours (AUC_{0-24}) of olanexidine and the metabolite DM-210 in serum. Serum C_{max} and AUC_{0-24} after repeated doses of olanexidine were higher in males than females. AUC_{0-24} in males tended to be higher after repeated doses than after the first dose on Day 1. C_{max} and AUC_{0-24} did not differ between animals receiving olanexidine gluconate and those receiving olanexidine hydrochloride.

Table 13. Pharmacokinetic parameters after 4-week repeated subcutaneous injections of olanexidine gluconate and olanexidine hydrochloride

Treatment	Dose (mg/kg/day)	No. of animals	Olanexidine				DM-210			
			C_{max} (ng/mL)		AUC_{0-24} (ng·h/mL)		C_{max} (ng/mL)		AUC_{0-24} (ng·h/mL)	
			Day 1	Last treatment day	Day 1	Last treatment day	Day 1	Last treatment day	Day 1	Last treatment day
Olanexidine gluconate	0.0278	9 males	0.415	0.684	1.50	5.20	BML	BML	-	-
		9 females	0.462	0.462	2.31	1.54	BML	BML	-	-
	0.278	9 males	9.65	8.30	48.3	75.5	BML	0.116	-	0.614
		9 females	4.92	5.09	35.6	44.4	0.086	0.155	0.130	0.269
	2.78	9 males	41.1	44.2	425	628	0.287	0.536	3.32	9.35
		9 females	34.7	35.7	355	407	0.486	1.25	6.01	13.6
Olanexidine hydrochloride	0.0272	9 males	0.551	0.765	2.47	4.77	BML	BML	-	-
		9 females	0.423	0.421	2.17	1.75	BML	BML	-	-
	0.272	9 males	9.92	8.67	47.0	72.7	BML	BML	-	-
		9 females	5.17	4.01	37.6	33.2	0.052	0.091	0.233	0.321
	2.72	9 males	49.2	40.5	465	517	0.322	0.571	3.14	6.79
		9 females	30.8	32.4	370	356	0.508	0.859	6.30	11.8

Mean; BML, below the lower limit of measurement (0.1 ng/mL); The minus (-) symbol indicates “not calculated.”

Table 14 summarizes serum C_{max} and AUC_{0-24} in dogs (4/sex/group) receiving subcutaneous injections of

olanexidine hydrochloride at 0.0109, 0.0544, or 0.272 mg/kg/day for 52 weeks. No sex differences were observed at any doses tested. AUC_{0-24} in the 0.272 mg/kg/day group was higher after repeated doses than after the first dose on Day 1.

Table 14. Serum pharmacokinetic parameters of olanexidine after 52-week repeated subcutaneous injections of olanexidine hydrochloride

Dose (mg/kg/day)	No. of animals	C_{max} (ng/mL)		AUC_{0-24} (ng·h/mL)	
		Day 1	Last treatment day	Day 1	Last treatment day
0.0109	4 males	0.80 ± 0.31	0.89 ± 0.50	7.82 ± 2.72	7.81 ± 3.43
	4 females	0.72 ± 0.16	0.52 ± 0.24	6.36 ± 0.83	5.46 ± 2.07
0.0544	4 males	4.46 ± 0.43	3.58 ± 0.61	37.4 ± 3.74	43.9 ± 5.10
	4 females	2.52 ± 0.81	2.98 ± 1.40	25.0 ± 6.56	38.3 ± 13.0
0.272	4 males	11.7 ± 3.50	12.4 ± 2.91	144 ± 22.9	231 ± 58.3
	4 females	7.24 ± 1.23	11.5 ± 2.75	112 ± 21.9	218 ± 32.0

Mean ± standard deviation

Five female rabbits received subcutaneous injections of olanexidine hydrochloride at 1.36 mg/kg/day for 13 days. C_{max} and AUC from 0 to 48 hours (AUC_{0-48}) in serum on Day 13 were as follows: olanexidine, 25.4 ng/mL (C_{max}) and 589 ng·h/mL (AUC_{0-48}); metabolite DM-210, 6.31 ng/mL (C_{max}) and 231 ng·h/mL (AUC_{0-48}); metabolite DM-210, 4.06 ng/mL (C_{max}) and 132 ng·h/mL (AUC_{0-48}).

Pregnant rabbits (4/dose) received subcutaneous injections of olanexidine hydrochloride at 0.0272, 0.272, or 1.36 mg/kg/day for 13 days from day 6 of gestation. C_{max} of olanexidine in serum on Day 13 were 0.350, 5.30, and 20.5 ng/mL in rabbits given 0.0272, 0.272, and 1.36 mg/kg/day, respectively. AUC_{0-24} of olanexidine in serum on Day 13 were 4.21, 74.0, and 338 ng·h/mL in rabbits given 0.0272, 0.272, and 1.36 mg/kg/day, respectively.

3.(ii).A.(1).3) Single-dose dermal studies (4.2.2.2-8 to 4.2.2.2-10)

Single doses of 0.695% solutions of ^{14}C-labeled olanexidine gluconate and ^{14}C-labeled olanexidine hydrochloride were applied to intact or abraded skin on the back of male rats (3/time point).[20] Table 15 summarizes the pharmacokinetic parameters of radioactivity and olanexidine in serum.

Table 15. Pharmacokinetic parameters of olanexidine after a single dermal application of ^{14}C-labeled olanexidine gluconate or ^{14}C-labeled olanexidine hydrochloride

	Treatment	Radioactivity			Olanexidine		
		C_{max} (ng eq./mL)	AUC [a)] (ng eq.·h/mL)	$t_{1/2}$ (h)[b)]	C_{max} (ng/mL)	AUC [a)] (ng·h/mL)	$t_{1/2}$ (h)[b)]
Intact skin	Olanexidine gluconate	4.4 (1h) 6.0 (6h)	73.7 131	24.4 (24-72h)	1.76	10.4 15.7	3.5 (24-72h)
	Olanexidine hydrochloride	33.3 (0.5h) 15.5 (8h)	263 363	22.4 (24-72h)	1.30	16.5 19.8	3.3 (24-72h)
Abraded skin	Olanexidine gluconate	50.5	417 656	1.2 (0.5-2h) 18.5 (4-24h)	11.9	142 192	1.5 (0.5-2h) 15.7 (4-24h)

[20] A 2.5 cm-square lint cloth containing 0.3 mL of each solution was applied to the skin and covered with occlusive dressing for 24 hours.

	Treatment	Radioactivity			Olanexidine		
		C_{max} (ng eq./mL)	AUC [a)] (ng eq.·h/mL)	$t_{1/2}$ (h)[b)]	C_{max} (ng/mL)	AUC [a)] (ng·h/mL)	$t_{1/2}$ (h)[b)]
				22.9 (24-72h)			13.2 (24-72h)
	Olanexidine hydrochloride	206	895 1238	1.1 (0.5-2h) 10.1 (4-24h) 30.3 (24-72h)	22.6	123 147	1.3 (0.5-2h) 6.6 (4-24h) 15.4 (24-72h)

Mean

a) The values in the upper line indicate AUC_{0-24}, and those in the lower line indicate AUC_{0-72}.

b) Olanexidine concentrations decreased in a polyphasic manner. The elimination half-life was calculated over the time periods indicated in the parentheses.

Single dermal doses of ^{14}C-labeled olanexidine gluconate 0.5%, 1%, 1.5%, and 2% solutions were applied to intact skin on the back of male rats (4/time point) to calculate the pharmacokinetic parameters of radioactivity and olanexidine in serum.[20] In rats given 0.5%, 1%, 1.5%, and 2% solutions, C_{max} of serum radioactivity were 5.02, 7.26, 9.88, and 20.4 ng eq./mL, respectively, AUC from 0 to 168 hours (AUC_{0-168}) of serum radioactivity were 54.2, 110, 193, and 246 ng eq.·h/mL, respectively, C_{max} of serum olanexidine were 0.06, 0.23, 0.83, and 1.23 ng/mL, respectively, and AUC_{0-168} of serum olanexidine were 0.2, 5.5, 11.0, and 24.1 ng·h/mL, respectively.

3.(ii).A.(1).4) *In vitro* studies (4.2.2.2-11)

Franz diffusion cells were used to apply ^{14}C-labeled olanexidine gluconate 0.695% solution to the skin of rats, pigs, and humans. The cumulative penetration rate after 24-hour application was 0.46% in rats, 1.89% in pigs, and 0.33% in humans. The absorption rate was 8.47% in rats, 12.4% in pigs, and 4.60% in humans.

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

3.(ii).A.(2).1) Protein binding (4.2.2.3-2)

The protein binding of ^{14}C-labeled olanexidine hydrochloride was ≥99% in rat and human sera at all concentrations evaluated (0.1 to 10 µg/mL).[21]

3.(ii).A.(2).2) Tissue distribution (4.2.2.2-2, 4.2.2.2-3, 4.2.2.2-5, 4.2.2.3-1)

Male and female rats (3/sex/time point) received a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg, and the tissue distribution of radioactivity was determined. In male rats, the radioactivity concentration peaked at 6 or 24 hours postdose in most tissues tested, and the radioactivity remained at 72 hours postdose in all tissues. At all time points[22], the tissue radioactivity concentration was highest at the administration site (ranged from 6827 to 56,282 ng eq./g), followed by the adrenal glands (1911 ng eq./g at 24 hours postdose), the thyroid gland (873 ng eq./g at 6 hours postdose), the kidneys (480 ng eq./g, at 6 hours postdose), the lungs (442 ng eq./g at 6 hours postdose). The peak radioactivity concentration in serum was 31.1 ng eq./mL at 2 hours postdose. Distribution of ^{14}C-labeled olanexidine in blood cells ranged between 41.1% and 63.7% during the period from 0.5 to 72 hours postdose. At 2 hours postdose, the

[21] Concentrations are expressed as values corresponding to olanexidine hydrochloride anhydride.

[22] The tissue radioactivity concentration was determined at 0.5, 2, 6, 24, and 72 hours postdose.

radioactivity concentrations in the ovary (68.6 ng eq./g) and uterus (24.1 ng eq./g) of female rats were similar or higher than the serum radioactivity concentration (23.1 ng eq./mL).

Three male dogs received a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg. The tissue radioactivity concentration at 168 hours postdose was high in the kidneys (1590 ng eq./g), thyroid gland (817 ng eq./g), pancreas (550 ng eq./g), pituitary gland (415 ng eq./g), and submandibular gland (387 ng eq./g). The serum radioactivity concentration at 168 hours postdose was 31.2 ng eq./mL. Distribution of ^{14}C-labeled olanexidine in blood cells ranged between 22.9% and 45.2% during the period from 0.25 to 168 hours postdose.

A single dermal dose of ^{14}C-labeled olanexidine gluconate 0.695% solution was applied to intact or abraded skin on the back of male rats (3/time point),[20] to determine the tissue distribution of radioactivity. After the application on intact skin, radioactivity concentration peaked at 6 or 24 hours postdose in most tissues tested, and the radioactivity remained at 72 hours postdose in all tissues. At all time points,[22] tissue radioactivity concentration was highest at the site of application (ranging from 17,990 to 34,933 ng eq./g), followed by the adrenal glands (319 ng eq./g[23] at 24 hours postdose), the thyroid gland (149 ng eq./g at 6 hours postdose), the kidneys (78.7 ng eq./g at 6 hours postdose), and brown adipose tissue (72.8 ng eq./g at 6 hours postdose). Tissue distribution of radioactivity applied to abraded skin was similar to that applied to intact skin. However, tissue concentration of radioactivity applied to abraded skin was higher than that applied to intact skin.

3.(ii).A.(2).3) Placental transfer (4.2.2.3-3)

Pregnant rats (2 animals [one at 12 days of gestation and another at 18 days of gestation]/time point) received a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg, and the tissue distribution of radioactivity in dams and fetuses was determined. In dams, radioactivity concentrations in the ovary and placenta were higher than those in the blood, but no radioactivity was detected in fetuses or amniotic fluid.

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

3.(ii).A.(3).1) Possible metabolic pathways

On the basis of the studies described in Sections "3.(ii).A.(3).2) *In vivo* metabolism" and "3.(ii).A.(3).3) *In vitro* metabolism," the metabolic pathways of olanexidine were estimated as below (see Figure 1).

[23] Mean of values from 2 animals

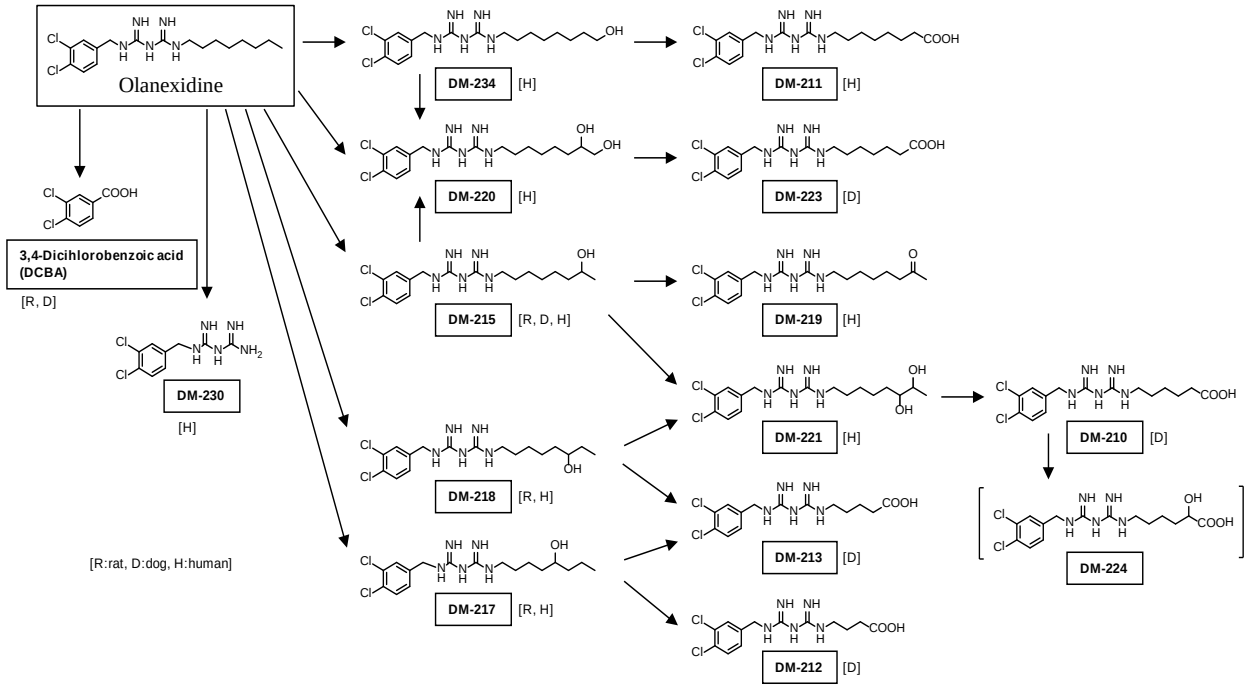

Figure 1. Possible metabolic pathways of olanexidine

3.(ii).A.(3).2) *In vivo* metabolism (4.2.2.2-4 to 4.2.2.2-6, 4.2.2.2-10)

Rats (3/sex/time point) received a single subcutaneous injection of olanexidine hydrochloride at 1360 mg/kg or repeated subcutaneous injections of 2.72 mg/kg/day for 11 days. Metabolites DM210 and DM212 were detected in serum.

Male rats (4/dose) received single dermal application of ^{14}C-labeled olanexidine gluconate 0.5% to 2% solutions to intact skin.[20] A metabolite of olanexidine (3,4-dichlorobenzoic acid [DCBA]) was detected in serum.[24]

Three male dogs received a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg, and metabolites in serum, urine, and feces were determined. DCBA was the major metabolite in serum. DM-213, DM-210, DM212, DM-223, and unknown metabolites were also detected in serum. DM-213, DCBA, DM-210, DM-212, and DM-223 were detected in urine. DM-213, DM-210, DM-223, DM-215, and unknown metabolites were detected in feces.

3.(ii).A.(3).3) *In vitro* metabolism (4.2.2.4-1 to 4.2.2.4-3)

Rat and human liver S9 fractions were used to investigate in vitro metabolism of olanexidine. DM-215, DM-217, and DM-218 were identified in the rat liver S9 fraction. This suggested the presence of metabolites

[24] No data were obtained for the concentrations of DM-210, DM-211, DM-212, DM-213, DM-223, DM-224, or DM240, as the measurement system was not considered robust as indicated by the results of assays with QC samples before and after the measurement of metabolites, which were outside the standard (with an accuracy of ± 15%).

containing a carbonyl group that results from oxidation of hydroxylated metabolites. In the experiment using human liver S9 fraction, DM-215, DM-217, DM-218, and DM-211 were identified.

Microsomes expressing human cytochrome P450 (CYP) were used to identify CYP isozymes that are involved in the metabolism of olanexidine. The results suggest the involvement of CYP2D6, CYP3A4, and CYP4F12.

Human liver microsomes and inhibitors of specific CYP isozymes were used to identify CYP isozymes involved in the metabolism of olanexidine. The results suggest the involvement of CYP2D6, CYP2E1, CYP3A4/5, and CYP4A/4F (CYP4F12).

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

3.(ii).A.(4).1) Excretion in urine, feces, and bile (4.2.2.2-2, 4.2.2.2-3, 4.2.2.2-5, 4.2.2.5-1, 4.2.2.5-2)

Three male rats, 3 female rats, and 3 male dogs received a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg. The radioactivity excretion rates in urine and feces for 168 hours postdose were 11.2% and 74.0%, respectively, in male rats, 13.4% and 65.2%, respectively, in female rats, and 49.9% and 32.0%, respectively, in dogs. Three male rats with bile duct cannulation received a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg; the radioactivity excretion rates in urine and bile were 13.1% and 43.0%, respectively.

Male rats (5/group) received a single dermal application of ^{14}C-labeled olanexidine gluconate 0.695% solution to intact skin or abraded skin.[20] The radioactivity excretion rates in urine and feces for 168 hours postdose were 0.6% and 2.1%, respectively, for intact skin, and 3.0% and 11.8%, respectively, for abraded skin.

3.(ii).A.(4).2) Excretion in milk (4.2.2.3-3)

In 3 female rats receiving a single subcutaneous injection of ^{14}C-labeled olanexidine gluconate at 1.39 mg/kg, the radioactivity concentration in milk peaked at 24 hours postdose. The peak radioactivity concentration in milk (41 ng eq./mL) was higher than the serum radioactivity concentration in dams (18 ng eq./mL).

3.(ii).A.(5) Pharmacokinetic interactions: enzyme inhibition and enzyme induction (4.2.2.6-1, 4.2.2.6-2)

Human liver microsomes were mixed with CYP isozymes to investigate the inhibitory effect of olanexidine hydrochloride on the CYP isozymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4). Olanexidine hydrochloride inhibited CYP2D6 (IC_{50}, 2.71 µmol/L) and CYP2B6 (IC_{50}, 9.72 µmol/L), but did not inhibit other CYP isozymes (IC_{50}, >10 µmol/L). Olanexidine hydrochloride inhibited CYP2B6 after a 30 minute incubation (IC_{50}, 4.57 µmol/L), showing time-dependent inhibition of CYP2B6. The inhibition constant (Ki) of olanexidine hydrochloride was 7.13 µmol/L for CYP2B6, 1.67 µmol/L for CYP2D6, and 6.86 µmol/L for CYP3A4. Olanexidine hydrochloride exhibited competitive inhibition of CYP2D6 and CYP3A4 and mixed inhibition of CYP2B6. The olanexidine hydrochloride

concentration that inhibited all CYP isozymes evaluated was higher than the peak serum olanexidine concentration observed in patients in a phase III clinical study (1.54 ng/mL; 0.0041 μmol/L) (see Section "4.(ii).A.(4) Phase III studies"). The applicant explained that olanexidine gluconate is thus unlikely to directly interact with drugs metabolized by CYP isozymes.

3.(ii).A.(6) Others

3.(ii).A.(6).1) Effects of surgical procedures on serum olanexidine concentration (4.2.2.7-1)

Olanexidine gluconate 1.5% solution was applied to the abdominal skin (approximately 15 cm × 25 cm) of minipigs (6 females and 4 males per group). After the application site was dried, the animals underwent laparoscopic surgery or laparotomy. Serum olanexidine concentrations were determined after surgery. The serum olanexidine concentration at 0.5 to 24 hours postdose ranged from 0.01 to 0.07 ng/mL for laparoscopic surgery and from 0.03 to 0.2 ng/mL for laparotomy. The applicant explained that the serum olanexidine concentration did not differ between the two different surgical procedures.

3.(ii).A.(6).2) Effects of the incision length on serum olanexidine concentration (4.2.2.7-2)

Olanexidine gluconate 1.5% solution was applied to the abdominal skin of male rats (6/group). After the application site was dried, laparotomy was performed with an incision length of 0, 1, 3, or 5 cm to evaluate the effects of the incision length on serum olanexidine concentration. The serum olanexidine concentration after laparotomy (0.5 to 3 hours postdose) by incision length ranged from 1.4 to 2.6 ng/mL for 0 cm, 1.6 to 3.8 ng/mL for 1 cm, 1.5 to 3.4 ng/mL for 3 cm, and 1.8 to 7.3 ng/mL for 5 cm. The applicant explained that the length of skin incision does not affect serum olanexidine concentration.

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

PMDA concluded that no specific problems were found in the results of non-clinical pharmacokinetic studies.

3.(iii) Summary of toxicology studies

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

The results of the following toxicology studies were submitted in the application: single-dose toxicity studies, repeated-dose toxicity studies, genotoxicity studies, reproductive toxicity studies, local tolerance studies, and other toxicity studies (skin phototoxicity studies, toxicity studies of impurities, and toxicity studies of metabolites). Olanexidine hydrochloride was used in all studies except for some studies (e.g. the local tolerance studies, skin phototoxicity studies, and skin photosensitization studies). The applicant explained that the effects of olanexidine gluconate can be evaluated using the results of toxicology studies of olanexidine hydrochloride, since the results of a 4-week repeated subcutaneous dose toxicity study of olanexidine gluconate 6.95% solution (4.2.3.7.4-1) indicate that olanexidine gluconate and olanexidine hydrochloride are similar in terms of toxicity and pharmacokinetic profiles.

Unless otherwise specified, olanexidine hydrochloride doses are expressed as equivalent doses of olanexidine

gluconate calculated based on the strength of olanexidine. Pharmacokinetic parameters of olanexidine hydrochloride are expressed as the free base of olanexidine. Unless otherwise specified, 5% D-mannitol aqueous solution was used as a vehicle.

3.(iii).A.(1) Single-dose toxicity (4.2.3.1-1 to 4.2.3.1-4)

SD rats (5/sex/group[25]) received a single subcutaneous injection of olanexidine hydrochloride at 1360 mg/kg[26] or a single oral dose of olanexidine hydrochloride at 170, 340, 680, 1360, and 2720 mg/kg,[26] No deaths occurred among animals receiving a subcutaneous injection. Animals receiving a subcutaneous injection exhibited salivation[27] and swelling at the administration site. These animals also showed a decrease in food consumption, a decrease in body weight or reduced body weight gain, and induration, hemorrhage, fistula formation and other findings at the administration site from the following day of administration. Among animals receiving an oral dose, deaths occurred in those given ≥680 mg/kg doses: 1 of 5 females in the 680 mg/kg group; 3 of 5 males and 1 of 5 females in the 1360 mg/kg group; and 1 of 5 males and 1 of 5 females in the 2720 mg/kg. These animals exhibited salivation, soft feces, abnormal respiratory sound, and a decrease in locomotor activities, among others.

Two male beagle dogs received a single subcutaneous injection of olanexidine hydrochloride at 1360 mg/kg.[26] No death occurred, and tremor[28] and swelling at the administration site were observed.

On the basis of these findings, the approximate lethal dose was determined to be >1360 mg/kg in rats given a subcutaneous injection, 680 to 1360 mg/kg in male rats given an oral dose, 340 to 680 mg/kg in female rats given an oral dose, and >1360 mg/kg in dogs given a subcutaneous injection.

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

Repeated-dose subcutaneous toxicity studies in rats (4- and 26-week studies) and dogs (4-, 13-, and 52-week studies) were conducted. Major toxicological findings observed both in rats and dogs included the effects on the administration site such as necrosis and fibrosis, extramedullary hematopoiesis in the spleen, and an increase in the production of granulocytic and myeloid cells in the bone marrow. The applicant explained that the systemic effects of olanexidine occurred secondary to the effect on the administration site. The no-observed-adverse-effect dose level (NOAEL) for systemic toxicity was 0.068 mg/kg/day in rats in the 26-week study and 0.0544 mg/kg/day in dogs in the 52-week study. The serum exposure to unchanged

[25] Oral doses of 170 and 340 mg/kg were administered only to 5 females each.

[26] An aqueous solution containing D-mannitol 5% and hydroxypropyl methylcellulose 1% was used as the vehicle.

[27] The applicant explained that salivation was probably not due to an effect of olanexidine on the central nervous system, considering the timing of onset of salivation (at 42 minutes to 2 hours postdose) and the pharmacokinetic findings that serum olanexidine concentration peaked at 8 hours postdose (T_{max}: 8 hours).

[28] The applicant's explanation: As olanexidine was found to distribute in the brain in dogs (see "3.(ii).A.(2).2) Tissue distribution"), the drug may have induced tremor by affecting the central nervous system. However, tremor is unlikely to develop in patients receiving the drug product in the clinical setting because the estimated serum exposure to the drug in animals with tremor (C_{max}, 35,750 ng/mL in males and 32,750 ng/mL females) is more than 100,000-fold the serum exposure in Study 131-301, a phase III study (C_{max}, 0.28 ng/mL).

olanexidine (AUC_{0-24}) at the NOAEL in rats and dogs was 11- to 23-fold and 11- to 13-fold higher, respectively, than the serum olanexidine exposure in patients in a clinical study.[29]

3.(iii).A.(2).1) Four-week repeated-dose subcutaneous toxicity study and 4-week recovery study in rats (4.2.3.2-1, 4.2.3.2-2)

SD rats (10/sex/group) received subcutaneous injections of olanexidine hydrochloride at 0 (physiological saline or vehicle), 0.0272, 0.272, and 2.72 mg/kg/day for 4 weeks. The rats were followed up for 4 weeks after the treatment to assess recovery. Animals in the ≥0.272 mg/kg/day groups exhibited extramedullary hematopoiesis in the spleen[30] and coagulative necrosis and granular tissue formation at the administration site. The 2.72 mg/kg/day group showed the following findings: decreases in food consumption and reduced body weight gain; decreases in red blood cell (RBC) count, hemoglobin, and other RBC parameters; increased reticulocyte count; increased white blood cell (WBC) count (mainly neutrophil count); increased platelet count; increases in serum total protein and aspartate aminotransferase[31]; decreases in lipids (i.e., cholesterol and phosopolipids), glucose, and calcium in serum; increases in the weight of the spleen and adrenal glands; enlargement of the spleen, adrenal glands, and axillary and submandibular lymph nodes; bone marrow discoloration; gross findings at the administration site (e.g., subcutaneous induration or white foci, scab, and hair loss); an increase in the production of granulocytes in the bone marrow; an increase in cell necrosis in the thymus; non-specific lymphadenitis in axillary lymph nodes; hypertrophy of the zona fasciculata of the adrenal glands; and calcium deposits at the administration site. These findings were reversed by the end of the 4-week recovery period. On the basis of the above findings, the NOAEL for systemic and local toxicity was determined to be 0.272 mg/kg/day and 0.0272 mg/kg/day, respectively.

3.(iii).A.(2).2) 26-week repeated-dose subcutaneous toxicity study (4.2.3.2-3, 4.2.3.2-4)

SD rats (10/sex in the control group, and 18/sex/group in the olanexidine hydrochloride groups) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0136, 0.068, and 0.68 mg/kg/day for 26 weeks. The ≥0.068 mg/kg/day groups showed induration and fibrosis at the administration site. The 0.68 mg/kg/day group showed the following findings: gross findings at the administration site (e.g., swelling, induration, scab, and hair loss); decreases in RBC count, hemoglobin, and other RBC parameters; increased reticulocyte count; increased WBC count (mainly neutrophil count); increased platelet count; an increase in serum alkaline phosphatase (ALP)[32]; change in serum protein level (e.g., increases in alpha- and beta-globulin,

[29] A comparison with the mean serum olanexidine exposure (AUC from 0 to 168 postdose, 3.323 ng·h/mL) in Study 131-301, a phase III study, was made.

[30] Extramedullary hematopoiesis in the spleen observed in the 0.272 mg/kg group was not considered as a systemic toxicity finding, as it was very slight in severity and was observed only in 1 of the 10 animals in association with inflammation at the administration site.

[31] The applicant explained that the increase in serum aspartate aminotransferase concentration was caused by the damage of subcutaneous muscle layer and skeletal muscle at the administration site rather than liver disorder, because animals exhibited coagulative necrosis in the subcutaneous tissue, subcutaneous muscle layer, and skeletal muscle, and granulation tissue formation accompanied by infiltration of inflammatory cells at the administration site, as well as a tendency toward an increase in serum creatine kinase level, while no histopathological changes were observed in the liver.

[32] The applicant explained that the increased ALP level in serum was probably unrelated to liver disorder for the following reasons: ALP level in serum increased in males, while lipid accumulation around hepatic lobules in the liver were found more often in females; no changes were observed in other markers of liver disorder; and no degenerative lesions were found in the liver.

and a decrease in albumin/globulin ratio [A/G ratio]); a decrease in serum lipids (cholesterol, phospholipids, and triglycerides); increases in the weight of the spleen, adrenal glands, and liver; a decrease in the weight of the thymus; coagulative necrosis and granular tissue formation at the administration site; extramedullary hematopoiesis in the spleen; non-specific lymphadenitis in axillary lymph nodes; an increase in the production of myelocytes in the bone marrow; and hepatocyte lipid droplet accumulation around hepatic lobules in the liver. On the basis of the above findings, the NOAEL for systemic and local toxicity was determined to be 0.068 mg/kg/day and 0.0136 mg/kg/day, respectively.

3.(iii).A.(2).3) Four-week repeated-dose subcutaneous toxicity study and 4-week recovery study in dogs (4.2.3.2-5)

Beagle dogs (4/sex in the control group and 3/sex/group in the olanexidine hydrochloride groups[33]) received subcutaneous injections of olanexidine hydrochloride at 0 (physiological saline or vehicle), 0.0109, 0.109, and 1.09 mg/kg/day for 4 weeks. The dogs were followed up for 4 weeks after treatment to assess recovery. The ≥0.109 mg/kg/day groups showed necrosis, inflammatory cell infiltration, and hemosiderin deposition at the administration site, and lymphadenitis in submandibular lymph nodes. The 1.09 mg/kg/day group showed the following findings: a decrease in food consumption; reduced body weight gain; gross findings at the administration site (e.g., swelling, induration, and adhesion to skeletal muscle), a decrease in urinary osmolality; increased WBC count (mainly neutrophil count); increased platelet count; change in serum protein levels (e.g., an increase in $\alpha_{2,3}$-globulin, a decrease in A/G ratio); a decrease in serum glucose level; an increase in serum lactate dehydrogenase; increases in the weight of adrenal glands and liver; a decrease in the weight of the thymus; thymus atrophy; an increase in the production of granulocytes in the bone marrow; extramedullary hematopoiesis in the spleen; atrophy of the thymic cortex; and fibrosis at the administration site. These findings were reversed by the end of the 4-week recovery period. On the basis of the above findings, the NOAEL for systemic and local toxicity was determined to be 0.109 mg/kg/day and 0.0109 mg/kg/day, respectively.

3.(iii).A.(2).4) Thirteen-week repeated-dose subcutaneous toxicity study and 4-week recovery study in dogs (4.2.3.2-6)

Beagle dogs (5/sex in the control group and 3/sex/group in the olanexidine hydrochloride groups) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0109, 0.0544, and 0.272 mg/kg/day for 13 weeks. The dogs were followed up for 4 weeks after treatment to assess recovery. The 0.0544 mg/kg/day group showed induration and yellow foci in the subcutaneous adipose tissue at the administration site and an increase in the formation of fibroblasts. The 0.272 mg/kg/day group showed the following findings: a decrease in body weight or reduced body weight gain, swelling at the administration site; increased WBC count (mainly neutrophil count); a decrease in thymus weight; an increase in serum ALP; change in serum protein levels (e.g., an increase in $\alpha_{2,3}$-globulin and β-globulin, and a decrease in A/G ratio); thymic atrophy;

[33] Five males and 5 females were used in the highest dose (1.09 mg/kg/day) group.

gelatinous subcutaneous adipose tissue, necrosis, and edema at the administration site. These findings were reversed by the end of the 4-week recovery period. On the basis of the above findings, the NOAEL for systemic and local toxicity was determined to be 0.0544 mg/kg/day and 0.0109 mg/kg/day, respectively.

3.(iii).A.(2).5) 52-week repeated-dose subcutaneous toxicity study in dogs (4.2.3.2-7)

Beagle dogs (4/sex/group) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0109, 0.0544, and 0.272 mg/kg/day for 52 weeks. The findings observed in the 0.272 mg/kg/day group were as follows: reduced body weight gain; increased WBC count (mainly neutrophil count); change in serum protein levels (e.g., a decrease in A/G ratio); lymphadenitis in superficial cervical lymph nodes; and the formation of yellow foci and necrosis of subcutaneous tissues at the administration site, an increase in the formation of fibroblasts and fibrosis, and inflammatory cell infiltration. On the basis of the above findings, the NOAEL for both systemic and local toxicity was determined to be 0.0544 mg/kg/day.

3.(iii).A.(3) Genotoxicity (4.2.3.3.1-1 to 4.2.3.3.1-3, 4.2.3.3.2-1)

The applicant conducted bacterial reverse mutation assays (Ames tests), a chromosomal aberration assay in Chinese hamster lung fibroblasts (CHL/IU cells), mouse lymphoma TK assay, and a micronucleus test in rats. The results of these studies were all negative. Olanexidine was thus considered to have no genotoxic potential.

3.(iii).A.(4) Reproductive toxicity

The applicant investigated the effects of olanexidine on fertility and early embryonic development in rats, embryo-fetal development in rats and rabbits, and pre- and postnatal development, including maternal function in rats. Olanexidine did not affect the fertility in male or female animals, early embryonic development, embryo-fetal development, or the development of offspring. The NOAEL for embryo-fetal toxicity was 2.72 mg/kg/day in rats and 1.36 mg/kg/day in rabbits. The serum olanexidine exposure[34] ($AUC_{0\text{-}48}$) at the NOAEL in rats and rabbits was approximately 170-fold and 177-fold higher, respectively, than the serum olanexidine exposure in patients in a clinical study.[29]

3.(iii).A.(4).1) Effects on fertility and early embryonic development in rats (4.2.3.5.1-1)

SD rats (25/sex/group) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0272, 0.272, and 2.72 mg/kg/day. The drug (or vehicle) was administered to male rats from 63 days before mating to the day before necropsy, and to female rats from 14 days before mating to day 7 of gestation. Male parent animals receiving 2.72 mg/kg/day exhibited decreased food consumption, reduced body weight gain, and spleen enlargement. Female parent animals receiving 2.72 mg/kg/day exhibited increased placental weight. Both male and female parent animals receiving 2.72 mg/kg/day exhibited induration, scab, hair loss, and subcutaneous hemorrhage at the administration site. No effects on fertility or early embryonic development

[34] As the serum exposure in pregnant rats was not assessed, the comparison was made with the serum exposure to unchanged olanexidine in the 11-day repeated-dose subcutaneous toxicity study in nonpregnant rats (4.2.2.2-6).

were observed. The applicant explained that the increased placental weight was not a significant toxicological finding because weight gain was very modest, with no effects on early embryonic development. On the basis of these findings, the NOAEL was determined to be 2.72 mg/kg for general (systemic) toxicity in female parent animals and for fertility and early embryonic development in male and female parent animals, and 0.272 mg/kg/day for general (systemic) toxicity in male parent animals and local toxicity in male and female parent animals.

3.(iii).A.(4).2) Effects on embryo-fetal development

(a) Studies in rats (4.2.3.5.2-1)

Pregnant SD rats (23 to 25/group) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0272, 0.272, and 2.72 mg/kg/day from days 7 to 17 of gestation. Dams receiving 2.72 mg/kg/day showed induration, scab, and subcutaneous hemorrhage at the administration site. A skeletal variation (an increased incidence of 14th rib) was observed in the embryos and fetuses of dams receiving 2.72 mg/kg/day. The applicant explained that this finding was not associated with olanexidine, because the incidence was within the range of historical data in the testing facility. On the basis of the above findings, the NOAEL was determined to be 2.72 mg/kg/day for general (systemic) toxicity in dams, fertility toxicity, and toxicity in embryos and fetuses, and 0.272 mg/kg/day for local toxicity in dams.

(b) Studies in rabbits (4.2.3.5.2-2)

Pregnant NZW rabbits (18 to 19/group) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0272, 0.272, and 1.36 mg/kg/day during the period from days 6 to 18 of gestation. Dams receiving 1.36 mg/kg/day showed decreased food consumption, reduced body weight gain, and subcutaneous hemorrhage at the administration site. No effects were observed in embryos or fetuses. On the basis of the above findings, the NOAEL was determined to be 0.272 mg/kg/day for general (systemic and local) toxicity in dams, and 1.36 mg/kg/day for toxicity in embryos and fetuses.

3.(iii).A.(4).3) Effects on pre- and postnatal development, including maternal function in rats (4.2.3.5.3-1)

Pregnant SD rats (19 to 20/group) received subcutaneous injections of olanexidine hydrochloride at 0 (vehicle), 0.0272, 0.272, and 2.72 mg/kg/day from days 7 to 21 of gestation. Dams receiving 2.72 mg/kg/day showed enlargement of the spleen[35] and induration, scab, hair loss, subcutaneous hemorrhage, and petechiae at the administration site. No effects were observed in the development or fertility of F1 offspring. On the basis of the above findings, the NOAEL was determined to be 2.72 mg/kg/day for general (systemic) toxicity in dams, toxicity on development and fertility of F_1 offspring, and toxicity in F_2 fetuses and 0.272 mg/kg/day for local toxicity in dams.

[35] Enlargement of the spleen was observed in 1 of the 20 animals. The applicant explained that this was a local effect of the test substance as this animal also exhibited induration at the site of administration.

3.(iii).A.(5) Local tolerance

3.(iii).A.(5).1) Primary skin irritation in rabbits (4.2.3.6-1, 4.2.3.6-2)

Male Kbl:JW rabbits (6/group) were exposed to 0.139%, 0.695%, and 6.95%[36] solutions of olanexidine gluconate or olanexidine gluconate that had undergone forced degradation[37] on intact skin and skin without stratum corneum without dressing. At 1, 24, and 72 hours, and 7 days postdose, the skin was evaluated by the Draize test. Skin irritation was caused by both olanexidine gluconate 6.95% solution and olanexidine gluconate 6.95% solution undergoing forced degradation. Skin without stratum corneum showed severer irritation than intact skin.

Male Kbl:JW rabbits (6/group) were exposed to olanexidine gluconate 0.139%, 0.278%, 0.417%, and 0.695% solutions, CHG 0.5% solution, and benzalkonium chloride (BKC) 0.2% solution on the skin of the back with occlusive dressing for 24 hours. At 24, and 72 hours, and 7 days postdose, the skin was evaluated by the Draize test. Olanexidine gluconate at ≥0.278% concentrations caused dose-dependent skin irritation. Skin irritating effect at 24 hours postdose was similar in animals receiving olanexidine gluconate 0.417% solution and those receiving BKC 0.2% solution. The skin irritation reaction to olanexidine gluconate was reversed by day 7 postdose. CHG 0.5% solution did not cause skin irritation.

Male Kbl:JW rabbits (6/group) were exposed to olanexidine gluconate 0.695% and 1.39% solutions, and CHG 4% solution on the intact skin and skin without stratum corneum of the back with occlusive dressing for 24 hours. At 24, 48, and 72 hours postdose, the skin was evaluated by the Draize test.[38] Olanexidine gluconate 1.39% solution was determined to be "slightly irritating," and CHG 4% solution "not irritating." The skin irritation of olanexidine gluconate did not increase in a concentration-dependent manner, nor did it differ between the intact and damaged skin.

3.(iii).A.(5).2) 14-day cumulative skin irritation in rabbits (4.2.3.6-3)

Male Kbl:JW rabbits (12/group [6 of 12 rabbits were assessed for recovery]) were exposed to olanexidine gluconate 0.695% and 1.39% solutions,[36] CHG 0.5% solution, and BKC 0.2% solution on the skin of the back without dressing once daily for 14 days. At 24 hours after each application, and 7 and 14 days after the last application of the test solutions, the skin was evaluated by the Draize test. Erythema was observed in animals receiving olanexidine gluconate 0.695% solution from day 8 onward and in animals receiving olanexidine gluconate 1.39% solution from day 3 onward. In animals receiving olanexidine gluconate 1.39% solution, the mean Draize score was highest on days 13 and 14 of treatment, and very mild irritation was also observed 7 and 14 days after the last administration. Olanexidine gluconate was thus considered to cause cumulative skin

[36] Sterile water for injection was used as the diluent for testing solutions and the vehicle control.

[37] Olanexidine gluconate 6.95% solution was kept at 60°C for 2 weeks.

[38] The primary skin irritation index (P.I.I.) was calculated using Draize scores at 24 and 72 hours postdose.

irritation. Histopathological examination at 1 day after the last administration revealed very mild or mild parakeratotic hyperkeratosis and acanthosis in animals receiving olanexidine gluconate 0.695% or 1.39% solution, and cellular infiltration in animals receiving olanexidine gluconate 1.39% solution. These findings were reversed within 14 days of the last administration. CHG 0.5% solution did not cause skin irritation. BKC 0.2% solution caused strong skin irritation. Olanexidine gluconate 0.695% and 1.39% solutions caused stronger skin irritation than CHG 0.5% solution and weaker skin irritation than BKC 0.2% solution.

3.(iii).A.(5).3) Eye mucous irritation in rabbits (4.2.3.6-1)

Olanexidine gluconate 0.139%, 0.695%, or 6.95% solution was placed into the conjunctival sacs of male Kbl:JW rabbits (3/group), and the upper and lower eyelids were held together for 30 seconds. The eyes were then rinsed with physiological saline (except for 3 animals receiving 0.139% solution and 3 animals receiving 0.695% solution). At 1, 24, 48, and 72 hours, and 7 and 14 days postdose, the eyes were evaluated by the Draize test.[39] The evaluation at 14 days postdose was made only for the animals exposed to the 6.95% solution. Olanexidine gluconate was determined to be "extremely slightly irritating" for the 0.139% solution with rinsing and "slightly irritating" for the 0.139% solution without rinsing, "slightly irritating" for the 0.695% solution with and without rinsing, and "moderately irritating" for the 6.95% solution. Animals receiving olanexidine gluconate 6.95% solution exhibited corneal opacity at 24 hours postdose and thereafter, and neovascularization in the peripheral part of the cornea on day 14 postdose. The above findings suggest that olanexidine gluconate 0.139%, 0.695%, and 6.95% solutions irritate the eye mucous membrane, and that olanexidine gluconate 6.95% solution severely irritates the eye mucous membrane.

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

3.(iii).A.(6).1) Antigenicity study in guinea pigs (4.2.3.7.1-1 to 4.2.3.7.1-3)

Male Hartley guinea pigs (10/group[40]) received subcutaneous and intramuscular injections of olanexidine hydrochloride at 0 (vehicle[36]), 0.139, 1.39, or 13.9 mg/kg or ovalbumin (OVA) at 1 mg/animal (the positive control), in combination with complete Freund's adjuvant, once weekly for 4 weeks. The animals were thus sensitized to the test substances. At 10, 14, and 15 days after the last dose, a challenging dose of olanexidine hydrochloride or OVA was administered to sensitized animals to induce active systemic anaphylaxis (ASA) reactions[41]. Animals receiving a challenging dose of olanexidine hydrochloride did not exhibit ASA reactions. Passive cutaneous anaphylaxis (PCA) was tested by exposing non-sensitized guinea pigs to serum samples obtained from sensitized guinea pigs. The non-sensitized guinea pigs showed no PCA reactions at 3 or 48 hours after exposure. On the basis of the above findings, olanexidine hydrochloride was determined not to be

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[39] As animals in the 6.95% solution group showed corneal opacity on day 7 postdose, the animals were observed again on day 14 postdose. At 72 hours postdose and thereafter, rabbit corneas were exposed to fluorescein to examine the corneal condition in detail. On the basis of the maximum Draize test score for each solution, the Key and Calandra classification (Calandra JC et al. *J Soc Cosm Chem*. 1962;13:281-289.) was used to classify the level of irritation.

[40] Serum samples for the studies on active systemic anaphylaxis and passive cutaneous anaphylaxis were obtained from 5 animals in each group.

[41] Animals sensitized to the vehicle or olanexidine hydrochloride were challenged with an intravenous administration of olanexidine hydrochloride at 6.8 mg/kg, and animals in the positive control were challenged with an intravenous administration of OVA 2 mg/animal.

antigenic. In another study, male Hartley guinea pigs (15/group) received subcutaneous and intramuscular injections of olanexidine hydrochloride at 0 (vehicle[36]), 1.39, and 13.9 mg/kg, or a conjugate of OVA with olanexidine hydrochloride ("olanexidine-OVA conjugate" hereinafter) at 1 mg/animal, in combination with complete Freund's adjuvant, once weekly for 4 weeks. These animals were thus sensitized to the test substances. At 12, 14, 15, or 16 days after the last dose, a challenging dose of olanexidine hydrochloride or olanexidine-OVA conjugate [42] was administered to sensitized animals to induce ASA reactions. Animals sensitized to olanexidine hydrochloride did not show ASA reactions. PCA was tested by exposing non-sensitized guinea pigs to serum samples obtained from sensitized guinea pigs. Two of 5 guinea pigs exposed to serum obtained from animals sensitized to the olanexidine-OVA conjugate exhibited weak PCA reactions 48 hours after receiving intravenous injection of olanexidine hydrochloride 6.8 mg/kg. The above findings suggest that olanexidine hydrochloride is weakly allergenic. Immunological cross-reactivity between olanexidine hydrochloride and chlorhexidine, an antiseptic similar to olanexidine, was investigated by testing PCA reactions: Serum samples obtained from animals sensitized to olanexidine-OVA conjugate and those from animals sensitized to DM-216-OVA conjugate[43] were used as testing sera. A conjugate of guinea pig serum albumin and olanexidine hydrochloride and a conjugate of guinea pig serum albumin and DM-216, were used as challenging antigens. Immunological cross-reaction was detected between olanexidine hydrochloride and chlorhexidine.

3.(iii).A.(6).2) Skin phototoxicity study (4.2.3.7.2-1)

Male Hartley guinea pigs (10 in the olanexidine groups, and 5 in the active control group) received olanexidine gluconate 0.695%, 1.39%, and 6.95% solutions, or the active control 8-methoxypsoralen on the skin of the back without dressing. The effects of these substances with and without ultraviolet irradiation[44] on the skin were evaluated.[45] Olanexidine gluconate at ≥1.39% concentrations caused skin reactions with a concentration-dependent increase in severity, but ultraviolet irradiation did not affect the degree of skin reactions. The results showed that olanexidine gluconate 6.95% solution does not cause skin phototoxicity.

3.(iii).A.(6).3) Skin sensitization test (4.2.3.7.3-1)

Male Hartley guinea pigs (10 each in the control and olanexidine groups, and 5 in the active control group) received an intradermal administration (primary sensitization) and a topical application (secondary sensitization) of olanexidine gluconate 6.95% solution on the skin of the back. The guinea pigs were thus sensitized to the test substance. At 21 days after the start of sensitization, olanexidine gluconate 0.695%, 1.39%, and 6.95% solutions were applied to the skin of the back for 24 hours with occlusive dressing. The

[42] Animals received intravenous administration of olanexidine hydrochloride at 6.8 mg/kg, or a conjugate of guinea pig serum albumin with olanexidine hydrochloride at 2 mg/animal.

[43] A conjugate of OVA with DM-216, a substance that consists of approximately half of the molecular structure of chlorhexidine with a carboxyl group.

[44] Animals in the irradiation groups were exposed to UVA at 10 joules/cm^2.

[45] The Morikawa method was utilized. The skin was assessed with the Draize test at 24, 48, and 72 hours after ultraviolet irradiation.

effects of the test substance on the skin were evaluated by the Draize test[46] at 24 and 48 hours after the removal of the dressing. No skin reactions were observed in animals receiving olanexidine gluconate. The results showed that olanexidine gluconate 6.95% solution does not cause skin sensitization.

3.(iii).A.(6).4) Skin photosensitization studies (4.2.3.7.3-2, 4.2.3.7.3-3)

Male Hartley guinea pigs (10 each in the control and olanexidine groups, and 5 in the active control group) were pretreated with olanexidine gluconate 6.95% solution for 5 days to induce photosensitivity. At 21 days after the initiation of the photosensitivity procedure, olanexidine gluconate 0.278% and 0.695% solutions were applied to the guinea pigs, and the skin was exposed to ultraviolet irradiation. The effects on the skin were evaluated by the Draize test[47] at 24 and 48 hours after ultraviolet irradiation. The skin reactions in the olanexidine gluconate groups were stronger than those in the control group, but did not markedly differ with or without ultraviolet irradiation. The skin reactions observed were considered to be caused by skin sensitization by olanexidine gluconate. Meanwhile, the skin sensitization test showed that olanexidine gluconate did not cause skin sensitization (4.2.3.7.3-1); this suggests that olanexidine gluconate 6.95% solution does not cause skin photosensitization but causes weak skin sensitivity. In another study using the Herber method, a method not using adjuvants, no skin reactions were observed in animals sensitized to olanexidine gluconate. The applicant explained that olanexidine gluconate is unlikely to cause skin sensitivity in the absence of adjuvants.

3.(iii).A.(6).5) 4-week repeated-dose subcutaneous toxicity studies of olanexidine gluconate and olanexidine hydrochloride in rats (4.2.3.7.4-1)

SD rats (10/sex/group) received subcutaneous injections of olanexidine gluconate at 0 (vehicle), 0.0278, 0.278, or 2.78 mg/kg/day, or olanexidine hydrochloride at 0.0278, 0.278, or 2.78 mg/kg/day for 4 weeks. Animals receiving olanexidine gluconate ≥0.0278 mg/kg/day exhibited the following findings: necrosis, infiltration of inflammatory cells, and fibrosis at the administration site; an increase in cell density in the bone marrow; and extramedullary hematopoiesis in the spleen. Animals receiving olanexidine gluconate 2.78 mg/kg/day exhibited the following findings: a decrease in food consumption; reduced body weight gain; decreases in RBC count, hematocrit, and other RBC parameters; increased reticulocyte count; increased WBC count (mainly neutrophil count); increased platelet count; decreases in serum protein (total protein and albumin), lipids (total cholesterol and phospholipids), and calcium; increases in serum creatinine and potassium; increases in the weight of the liver, spleen and adrenal glands; gross findings at the administration site (induration, scab, and white foci); histiocytosis in axillary lymph nodes; hypertrophy of the zona fasciculata of the adrenal glands; increased macrophage count in the thymus; and vacuolization of hepatocytes and

[46] The guinea pig maximization test was used. Animals in the control group were sensitized to sterile water for injection, and those in the active control group received 1-Chloro-2, 4-dinitrobenzene 0.1% solution in olive oil.

[47] The adjuvant and strip method was used. In the step of photosensitization, animals in the control group received sterile water for injection, those in the active control group received 6-methylcoumarin 2% solution. In the photosensitization challenge step, animals in the active control group received the 2% 6-methylcoumarin solution.

basophilic hepatocytes. These findings are consistent with the toxicity profile of olanexidine hydrochloride at the same dose (2.78 mg/kg/day). The serum exposure to olanexidine and its metabolite DM210 did not differ between animals receiving olanexidine gluconate and those receiving olanexidine hydrochloride.

3.(iii).A.(6).6) Toxicity studies of impurities (4.2.3.7.5-1 to 4.2.3.7.5-4)

The applicant conducted a bacterial reverse mutation test, a chromosomal aberration assay in CHL/IU cells, and a micronucleus test in mice to evaluate genotoxicity of an impurity (Impurity A) in olanexidine gluconate 2% solution, because the level of Impurity A in olanexidine gluconate 2% solution exceeded the threshold (0.15%) defined in the "Revision of the Guidelines on Impurities in New Drug Products" (PFSB/ELD Notification No. 0624001 of the Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, the Ministry of Health, Labour and Welfare, dated June 24, 2003). In the chromosomal aberration assay, chromosomal aberrations (numerical aberrations) were observed after 24-hour and 48-hour treatment, while micronucleus induction was not observed in the micronucleus test.[48] These results showed that Impurity A does not induce chromosomal aberrations[49] [redacted]

3.(iii).A.(6).7) Toxicity studies of metabolites (4.2.3.7.6-1, 4.2.3.7.6-2)

Major metabolites of olanexidine in humans (DM-210 and DM-212) were assessed for genotoxicity, general toxicity, and reproductive toxicity. Ames tests revealed that neither DM-210 nor DM-212 were genotoxic. A single subcutaneous injection of olanexidine hydrochloride at 1360 mg/kg[50] was administered to rats, and repeated subcutaneous injections of olanexidine hydrochloride at 1.36 mg/kg/day were administered to rabbits for 13 days[51] (4.2.2.2-4 and 4.2.2.2-7), to determine serum concentrations of metabolites of olanexidine. The serum exposure to DM-210 in rats and rabbits was 7.4-fold and 33-fold higher, respectively, than the serum exposure in patients in a clinical study.[52] The serum exposure to DM-212 in rats and rabbits was 1.1-fold and 17-fold higher, respectively, than the serum exposure in patients in a clinical study. It was thus determined that the genotoxicity, general toxicity, and reproductive toxicity of these metabolites were sufficiently assessed in these study systems.

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48 Mice received olanexidine gluconate 2% solution at 0 (0.5 w/v% methyl cellulose as a vehicle), 125, 250, and 500 mg/kg/day.

49 The mean plasma concentrations of Impurity A in the 500 mg/kg group at 1, 2, 4, and 24 hours after the last administration was ≥50 μg/mL, and the plasma exposure (C_{max}) at T_{max} (4 hours after the last administration) was 56.43 μg/mL. The applicant explained that the bone marrow was sufficiently exposed to Impurity A in this study system.

50 This dose corresponds to the highest doses in the single-dose subcutaneous study and the micronucleus test.

51 This dose corresponds to the highest dose in the embryonic/fetal toxicity study in rabbits.

52 Serum exposure in animals were compared with the serum exposure (AUC from 0 to 168 hours) to DM-210 (18.6 nmol·h/L) and DM-212 (22.8 nmol·h/L) in subjects receiving olanexidine gluconate 1.5% solution in Study 131-301.

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

Effects on the administration site

The effects of olanexidine on the administration site (e.g., subcutaneous necrosis, inflammation, and fibrosis) were observed in the repeated-dose subcutaneous toxicity studies. Further, the NOAEL for local toxicity was 0.0136 mg/kg/day in rats receiving 26 week-treatment and 0.0544 mg/kg/day in dogs receiving 52 week-treatment. The 0.0136 mg/kg/day and 0.0544 mg/kg/day correspond to olanexidine concentration of 0.0004 w/v% and 0.002 w/v%, respectively. These concentrations (0.0004 w/v% and 0.002 w/v%) are substantially lower than the concentration of olanexidine gluconate in Olanedine (1.5 w/v%). PMDA asked the applicant whether this finding may cause a safety concern in the clinical use of Olanedine.

The applicant's explanation:

The effects of olanexidine gluconate on the administration site (subcutaneous tissues) observed in the repeated-dose subcutaneous toxicity studies cannot be compared with those of other antiseptics currently available in the market (e.g., CHG, BKC), because the data on subcutaneous toxicity of these antiseptics have not been published. However, the proposed indication of olanexidine gluconate 1.5% solution is "preoperative skin preparation at the surgical site," and the solution is to be applied once to the skin. Although local toxicity was observed in the non-clinical studies, the route of administration and treatment regimen (repeated subcutaneous injections) used in the non-clinical studies are different from those to be used in the clinical setting (single application to skin). Thus, the data in the non-clinical studies cannot be directly compared with the effects in the clinical use, because the site and duration of administration differ between the non-clinical studies and the clinical use. In Study 131-301, a phase III study, olanexidine and its metabolites were found in serum samples from some patients. In these patients, olanexidine gluconate applied on the skin was probably transferred into the body through surgical procedures including the following: one or multiple skin incisions; insertion of access ports through the skin incision; surgical steps from the insertion of instruments through the ports to the resection of internal organs; procedures associated with internal or external anastomosis; and wound suturing. In the clinical studies of olanexidine gluconate, dermal findings at the administration site (application site erythema, application site pruritus, and application site dermatitis) were reported as adverse events for which causal relationship to the study drug could not be ruled out. These findings were not serious and were reversible.

Local toxicity was caused by repeated subcutaneous injections in toxicity studies, but this treatment regimen will not be used in the clinical setting. This fact and safety data from the clinical studies suggest that the subcutaneous findings from the repeated dose toxicity studies are unlikely to pose safety concerns regarding the clinical use of Olanedine.

PMDA's view:

Human subcutaneous tissues may be damaged if exposed to olanexidine gluconate used in the clinical setting. However, olanexidine gluconate is unlikely to penetrate into subcutaneous tissues in humans after application

of Olanedine (the 1.5 w/v% solution) to the skin, because Olanedine is applied once to the skin and left to dry in open air. Further, the skin irritation studies in rabbits (4.2.3.6-2 and 4.2.3.6-3) did not reveal a substantial difference in skin irritability between olanexidine gluconate and conventional antiseptics. Thus the subcutaneous findings observed after the application of olanexidine on intact skin are unlikely to pose safety concerns in the clinical setting.

The toxicity studies conducted in the United States revealed toxicity findings (i.e., vacuolization of hepatocytes, increased WBC count, and increased production of myeloid and granulocytic cells), raising safety concerns before phase I clinical studies were started in the United States (see "1. Origin or history of discovery, use in foreign countries, and other information"). PMDA considers that these toxicity findings are toxicologically insignificant, because no similar findings were obtained in the 4-week repeated-dose subcutaneous toxicity study or 4-week recovery study in rats (4.2.3.2-1, and 4.2.3.2-2) or the 4-week repeated-dose subcutaneous toxicity study or 4-week recovery study in dogs (4.2.3.2-5), despite that these repeated-dose toxicity studies were designed to achieve higher systemic exposure as compared to that in dermal application.[53] (The results of these studies are included in the evaluation data for regulatory review in Japan.) Meanwhile, systemic effects were observed in the Japanese repeated-dose subcutaneous toxicity studies in rats and dogs (e.g., extramedullary hematopoiesis in the spleen, increased WBC count, and increased production of granulocytic/myeloid cells in the bone marrow). The applicant explained that these systemic effects were secondary changes associated with the local effects on the administration site; this explanation is understandable. The NOAEL for systemic toxicity was 0.068 mg/kg/day in rats in the 26-week study and 0.0544 mg/kg/day in dogs in the 52-week study; the serum exposure to unchanged olanexidine ($AUC_{0\text{-}24}$) at the NOAEL in rats and dogs was 11- to 23-fold and 11- to 13-fold higher, respectively, than the serum olanexidine exposure in patients in a clinical study.[54] PMDA thus considers that these systemic effects are toxicologically insignificant.

4. Clinical data

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

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

No results of biopharmaceutical studies were submitted in this application.

Concentrations of olanexidine and its metabolites (i.e., DM-210, DM-211, DM-212, DM-213, DM-223, DM-224, and 3, 4-dichlorobenzoic acid [DCBA]) in human serum and urine were determined by liquid chromatography-tandem mass spectroscopy. The lower limit of quantification (LLOQ) for serum was as follows: 0.05 ng/mL for olanexidine, DM-210, DM-211, DM-212, DM-213, and DM-223; 0.1 ng/mL for

[53] No systemic effects, including hepatotoxicity, were observed in 4-week repeated-dose subcutaneous toxicity studies in rabbits or minipigs that were additionally conducted in the United States.

[54] A comparison with the mean serum olanexidine exposure (AUC from 0 to 168 postdose, 3.323 ng·h/mL) from Study 131-301, a phase III study, was made.

DM-224; and 0.5 ng/mL for DCBA. The LLOQ for urine was 0.05 ng/mL for olanexidine and 0.5 ng/mL for metabolites.

4.(ii) Summary of clinical pharmacology studies

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

The results of 2 phase I studies, a phase I/II study, and a phase III study in Japan were submitted. The results of *in vitro* studies using human biological samples are described in Sections "3.(ii).A.(2) Distribution," "3.(ii).A.(3) Metabolism," "3.(ii).A.(5) Pharmacokinetic interactions: enzyme inhibition and enzyme induction."

4.(ii).A.(1) Phase I single-dose study (5.3.3.1-1, Study 131-102 [[redacted] to [redacted]])

A total of 47 healthy adults received a single-dose application of 3 mL of olanexidine gluconate 0.278%, 0.695%, or 1.39% solutions to a 130 cm^2 area of the skin on the abdomen (n=23) or the inguinal region (n=24).[55] The concentrations of olanexidine and its metabolites in serum and urine were then determined. (These 47 subjects were included in the pharmacokinetics data set [PKS].) The concentrations of olanexidine and its metabolites in serum and urine were below the LLOQ at all time points in all subjects.

4.(ii).A.(2) Phase I single-dose study (5.3.3.1-2, Study 131-104 [[redacted] to [redacted]])

A total of 39 healthy adults received a single-dose application of olanexidine gluconate 1.5% solution to the skin on the right and left sides of the abdomen (130 cm^2 on each side [260 cm^2 in total]) and to the skin on the right and left sides of the inguinal region (30 cm^2 on each side [60 cm^2 in total]).[56] The concentrations of olanexidine and its metabolites in serum were then determined. (These 39 subjects were included in the PKS.) The concentrations of olanexidine and its metabolites in serum were below the LLOQ at all time points in all subjects.

4.(ii).A.(3) Phase I/II single-dose study (5.3.5.1-2, Study 131-202 [[redacted] to [redacted]])

A total of 27 healthy adults received a single-dose application of olanexidine gluconate 1%, 1.5%, or 2% solutions to the skin on the right and left sides of the abdomen (130 cm^2 on each side [260 cm^2 in total]) and to the skin on the right and left sides of the inguinal region (30 cm^2 on each side [60 cm^2 in total]).[56] The concentrations of olanexidine and its metabolites in serum were then determined. (These 27 subjects were included in the PKS.) In 1 subject each in the 1% and 1.5% groups, olanexidine was found in serum at 1 hour postdose at concentrations of 0.276 ng/mL (the 1% group) and 0.136 ng/mL (the 1.5% group). In both subjects, however, serum olanexidine concentration was below the LLOQ at the other time points. In all subjects in the 2% group, serum olanexidine concentration was below the LLOQ at all time points. The serum concentrations of metabolites were below the LLOQ at all time points in all subjects.

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[55] The olanexidine gluconate solution was dripped onto the skin area repeatedly to spread then allowed to dry. After 72 hours, the solution was removed.

[56] The study drug was applied on each skin area for 2 minutes then allowed to dry in open air.

4.(ii).A.(4) Phase III study (5.3.5.1-4, Study 131-301 [[redacted], [redacted] to [redacted], [redacted]])

A total of 52 patients scheduled for laparoscopic surgery for the treatment of gastrointestinal disorder received olanexidine gluconate 1.5% solution on the skin at the surgical site,[57] then the concentrations of olanexidine and its metabolites in serum were determined. These 52 patients were included in the PKS. In 46 patients, olanexidine or metabolites were detected in serum. In 25 patients, olanexidine was detected in serum at 0.5 to 3 hours after the application of the olanexidine gluconate solution, and the olanexidine concentration decreased below the LLOQ by 168 hours after application. The mean peak serum concentration of olanexidine (C_{max}) in these patients was 0.280 (0.053-1.54) ng/mL, and the mean area under the concentration versus time curve of olanexidine from zero to time t[58] (AUC_{0-t}) was 3.32 (0.583-23.2) ng·h/mL. The following metabolites were found in the serum: DM-210 in 36 patients, DM-211 in 16 patients, DM-212 in 41 patients, and DM-213 in 10 patients. Serum concentration of each metabolite in these patients ranged from 0.051 to 0.787 ng/mL for DM-210, 0.050 to 0.149 ng/mL for DM-211, 0.050 to 1.058 ng/mL for DM-212, 0.053 to 0.105 ng/mL for DM-213. Concentrations of DM-223, DM-224, and DCBA were below the LLOQ at all time points in all subjects.

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

Systemic exposure to olanexidine and its metabolites

In the phase I studies (Studies 131-102 and 131-104) and the phase I/II study (Study 131-202) conducted in healthy adults, serum concentrations of olanexidine and its metabolites were below the LLOQ in almost all subjects. In the phase I/II study (Study 131-202), serum olanexidine concentrations exceeded the LLOQ in 2 of 27 subjects. In the phase III study conducted in patients scheduled for laparoscopic surgery (Study 131-301), the serum concentrations of olanexidine or metabolites exceeded the LLOQ in 46 of 52 patients and differed among patients substantially. PMDA thus asked the applicant to explain the reasons the transdermal absorption of olanexidine differed between healthy adults and patients scheduled for laparoscopic surgery as well as the safety of olanexidine in patients with high serum concentrations of olanexidine and/or metabolites.

The applicant's explanation:

In the phase I/II study (Study 131-202), baseline characteristics (age, height, weight, complications, the dose of the drug, and skin condition) did not differ substantially between the 2 subjects with a serum olanexidine concentration above the LLOQ and the other 25 subjects with a serum olanexidine concentration below the LLOQ. Thus, no factors that could explain the difference in the transdermal absorption of olanexidine among healthy individuals were found.

Olanexidine and/or its metabolites were detected in the serum of many patients in a phase III study (Study

[57] The site of application was allowed to dry without dressing before surgery.

[58] The time point next to the last time point at which serum olanexidine concentration exceeded the limit of detection. However, when serum olanexidine concentration exceeded the limit of detection at 168 hours postdose, the t value was set at 168 hours.

131-301). In these patients, olanexidine solution applied to the skin was probably transferred into the body through skin incision and surgical procedures, resulting in the detection of olanexidine and/or its metabolites in serum. The differences in the serum concentrations of olanexidine and its metabolites among individual patients in the phase III study (Study 131-301) may be due to differences in diverse factors, including the amount of the drug applied, the time from the end of the drug application to the start of surgery, duration of surgery, the amount of bleeding during surgery, and type of surgery (single incision laparoscopic surgery vs. other procedures). These factors may have affected the amount of olanexidine distributed into the body.

The serum concentrations of olanexidine and its metabolites differed substantially among patients who received the drug before laparoscopic surgery, but were not related to the incidence of adverse events. The adverse events (application site erythema in 2 patients, blood pressure decreased in 2 patients, and nausea, vomiting, and erythema in 1 patient each) that developed in subjects with a relatively high olanexidine concentration in serum (C_{max} ≥0.300 ng/mL) were not related to olanexidine gluconate. Thus, systemic exposure to olanexidine and its metabolites is unlikely to affect the safety of olanexidine gluconate in the clinical setting.

PMDA's view:
In the phase III study (Study 131-301), no association was observed between the serum concentrations of olanexidine or its metabolites and the incidence of adverse events, and no safety concerns were raised in subjects with relatively high serum concentrations of olanexidine or its metabolites. However, currently available data are limited to those obtained from a small number of patients scheduled for laparoscopic surgery for the treatment of gastrointestinal disorder. Further, the applicant explained that the difference in the serum concentrations of olanexidine and its metabolites among individuals may be related to diverse factors such as the amount of olanexidine gluconate 1.5% solution applied, the time from the completion of application to the start of surgery, duration of surgery, the amount of bleeding during surgery, and type of surgery (single incision laparoscopic surgery vs. other procedures). The limited available data and the applicant's explanation suggest that serum olanexidine concentration may become higher in patients undergoing surgery in other regions or with different procedures than in the patients evaluated in the clinical study (Study 131-301) who underwent laparoscopic surgery. Accordingly, the safety of olanexidine gluconate in preoperative skin preparation for other surgical regions and procedures should be evaluated in the post-marketing settings. The safety of Olanedine (olanexidine gluconate 1.5% solution) is described in Section "4.(iii).B.(2) Safety."

4.(iii) Summary of clinical efficacy and safety

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

The applicant submitted evaluation data regarding the efficacy and safety of Olanedine (the results of two phase I studies, a phase I/II study, a phase II study, and two phase III studies conducted in Japan) and reference data (the results of a phase I study and a phase II study conducted in foreign countries). Table 16

shows the outline of clinical study data submitted to PMDA.

Table 16. Outline of clinical studies (evaluation data)

Phase	Study No.		Subjects	Dosage regimen		No. of subjects	Purpose
I	131-102		Healthy adult men	Olanexidine gluconate 0.278%, 0.695%, 1.39%	A single dose dripped onto the skin of the abdomen or inguinal region	48	Safety, Pharmacokinetics
I	131-104		Healthy adult men	Olanexidine gluconate 1.5%, Vehicle	Single application on the skin of right and left sides of the abdomen and inguinal region	59	Pharmacokinetics, Safety
II	131-201		Healthy adults	Olanexidine gluconate 0.278%, 0.695%, 1.39%, PVI 10%, CHG 0.5%, Physiological saline	Single application on the skin of the abdomen or inguinal region	160	Efficacy, Safety
I /II	131-202	Study 1	Healthy adult men	Olanexidine gluconate 1%, 1.5%, 2%, Physiological saline, Vehicle	Single application on the skin of right and left sides of the abdomen and inguinal region	63	Safety, Pharmacokinetics
		Ex vivo study	Healthy adult men	Olanexidine gluconate 2%, PVI 10%, CHG 0.5%	Single application on the skin of the left side of the abdomen	15	Inactivation by an inactivating agent
		Study 2	Healthy adults	Olanexidine gluconate 1%, 1.5%, 2%, PVI 10%, CHG 0.5%, Physiological saline	Single application on the skin of right and left sides of the abdomen and inguinal region	216	Efficacy, Safety
III	131-301		Patients scheduled for laparoscopic surgery	Olanexidine gluconate 1.5%, PVI 10%	Single application on the skin at the surgical site	106	Safety, Pharmacokinetics
III	131-302		Healthy adults	Olanexidine gluconate 1.5%, CHG 0.5%, Vehicle	Single application on the skin of right and left sides of the abdomen and inguinal region	594	Efficacy, Safety

PVI, povidone iodine solution; CHG, chlorhexidine gluconate

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

4.(iii).A.(1).1) Phase I study (5.3.3.1-1, Study 131-102 [[redacted], [redacted] to [redacted], [redacted]])

An open-label, non-controlled study in healthy adult men (target sample size, 48) was conducted at a study site in Japan to assess the safety and pharmacokinetic profile of solutions containing olanexidine gluconate (see Section "4.(ii).A.(1) Phase I single-dose study").

In this study, 3.0 mL of olanexidine gluconate 0.278%, 0.695%, or 1.39% solution was dripped onto a 130 cm^2 area of the abdominal or inguinal skin then allowed to dry. The drug was removed after 72 hours.

All 48 subjects receiving olanexidine gluconate solutions were included in the safety analysis set. The 48 subjects consist of 16 subjects each receiving application of 0.278%, 0.695%, or 1.39% solutions to the skin of the abdomen (8 of the 16 subjects) or inguinal region (the other 8 of the 16 subjects).

Adverse events, including abnormal laboratory values, developed within 72 hours postdose in 8 of 8 subjects receiving the 0.278% solution to the abdominal skin, 7 of 8 subjects receiving the 0.278% solution to the inguinal skin, 8 of 8 subjects receiving the 0.695% solution to the abdominal skin, 8 of 8 subjects receiving the 0.695% solution to the inguinal skin, 8 of 8 subjects receiving the 1.39% solution to the abdominal skin, and 8 of 8 subjects receiving the 1.39% solution to the inguinal skin. Adverse events observed in ≥ 2 subjects

in at least one group are listed in Table 17.

Table 17. Adverse events developing in ≥2 subjects in at least one group (Safety analysis set)

Adverse events	Olanexidine gluconate 0.278% solution		Olanexidine gluconate 0.695% solution		Olanexidine gluconate 1.39% solution	
	Abdominal skin	Inguinal skin	Abdominal skin	Inguinal skin	Abdominal skin	Inguinal skin
No. of subjects	8	8	8	8	8	8
No. of subjects with adverse events	8 (100)	7 (87.5)	8 (100)	8 (100)	8 (100)	8 (100)
Albumin globulin ratio increased	1 (12.5)	0	0	0	0	2 (25.0)
Blood LDH decreased	1 (12.5)	1 (12.5)	4 (50.0)	4 (50.0)	5 (62.5)	5 (62.5)
Blood potassium increased	2 (25.0)	1 (12.5)	0	2 (25.0)	1 (12.5)	1 (12.5)
Body temperature decreased	3 (37.5)	4 (50.0)	4 (50.0)	5 (62.5)	3 (37.5)	2 (25.0)
Haematocrit decreased	2 (25.0)	0	0	0	2 (25.0)	0
Haemoglobin decreased	1 (12.5)	0	0	0	2 (25.0)	0
Protein total decreased	4 (50.0)	1 (12.5)	4 (50.0)	4 (50.0)	7 (87.5)	3 (37.5)
Eosinophil percentage increased	1 (12.5)	0	2 (25.0)	3 (37.5)	2 (25.0)	0
Application site erythema	0	0	2 (25.0)	1 (12.5)	2 (25.0)	1 (12.5)
Erythema	5 (62.5)	2 (25.0)	0	0	0	0
Urticaria	3 (37.5)	1 (12.5)	0	0	0	0

No. of subjects (%)
LDH, lactate dehydrogenase

No deaths or serious adverse events were observed. Adverse events resulting in the discontinuation of study treatment were limited to an event of conjunctival hyperaemia in 1 subject receiving olanexidine gluconate 1.39% solution to the abdominal skin. A causal relationship between the event and olanexidine gluconate was ruled out. The outcome of the event was "recovered/resolved."

4.(iii).A.(1).2) Phase I study (5.3.3.1-2, Study 131-104 [[redacted], [redacted] to [redacted], [redacted]])

A randomized, double-blind, parallel-group study in healthy adult men (target sample size, 60 [40 in the olanexidine gluconate group and 20 in the vehicle control group]) was conducted at a study site in Japan to assess the pharmacokinetic profile and safety of olanexidine gluconate 1.5% solution (see Section "4.(ii).A.(2) Phase I single-dose study").

In this study, the subjects received a 2-minute single application of olanexidine gluconate 1.5% solution or placebo (vehicle) to the skin on the right and left sides of the abdomen (130 cm^2 on each side [260 cm^2 in total]) or the skin on the right and left sides of the inguinal region (30 cm^2 on each side [60 cm^2 in total]).[59] The applied solutions were then allowed to dry without dressing.

All 59 subjects receiving the study drug (39 receiving olanexidine gluconate 1.5% solution and 20 receiving the vehicle solution) were included in the safety analysis set.

Adverse events, including abnormal laboratory values, developed by 72 hours postdose in 13 of 39 subjects (33.3%) in the olanexidine gluconate 1.5% group and 4 of 20 subjects (20.0%) in the vehicle control group. All of these adverse events were "protein total decreased." A causal relationship to study treatment was ruled

[59] The study drug applied was kept on the skin until the end of observation and examination at 72 hours postdose.

out in all events. No deaths, serious adverse events, or adverse events resulting in the discontinuation of study treatment were observed.

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

4.(iii).A.(2).1) Phase II study (5.3.5.1-1, Study 131-201 [[redacted], [redacted] to [redacted], [redacted]])

A randomized, open-label,[60] parallel-group study was conducted in healthy adults at 3 study sites in Japan to assess the efficacy and safety of single application of olanexidine gluconate solutions versus placebo, PVI 10% solution, and CHG 5% solution. The target sample size was 162 (78 receiving the study drug to the abdominal skin [n=13 per group] and 84 receiving the study drug to the inguinal skin [n=14 per group]).[61]

In this study, olanexidine gluconate 0.278%, 0.695%, or 1.39% solution, PVI 10% solution, CHG 0.5% solution, or physiological saline was applied to the abdominal or inguinal skin for 2 minutes using a cotton swab, and the skin was allowed to dry without dressing. Samples for bacteriological testing were obtained from the abdominal or inguinal skin at baseline and 10 minutes and 6 hours after application of study drug,[62] to determine viable bacterial count.[63] Of 162 randomized subjects, 160 subjects[64] who received the study drug were included in the safety analysis set. These 160 subjects were included in the per-protocol set (PPS) for efficacy analysis.

Table 18 summarizes the results of primary endpoints: the decrease in viable bacterial count from baseline and its difference between active agents and saline.

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60 As the study drugs used in the study had different colors, blinding to the study subjects could not be achieved. However, individuals who performed skin sampling for viable bacterial count or microbial testing in the central laboratory were blinded for study drug allocation.

61 Individuals who satisfied the following criteria for viable bacterial count at screening were enrolled in the study: ≥2.0 $Log_{10}CFU/cm^2$ on the abdominal skin, or ≥4.0 $Log_{10}CFU/cm^2$on the inguinal skin.

62 The study drugs were applied to the skin on the right and left sides of the abdomen and inguinal region. Each skin area was divided into 4 compartments: Abdominal skin was divided horizontally and longitudinally into four compartments by two perpendicular lines, while the inguinal skin was divided longitudinally into four. Screening bacteriological testing was performed using samples from the left-most compartment of the left inguinal skin area and the upper-left compartment of the left abdominal skin area. The remaining 3 compartments of the left abdominal and inguinal skin areas were kept as backup. Three of the four components of the right abdominal and inguinal skin areas were treated with the allocated study drug, and samples for bacteriological testing were obtained at baseline, 10 minutes, or 6 hours after application. The remaining 1 compartment was kept as backup.

63 The study drug applied to the skin area was kept on the skin until the end of sampling for bacteriological testing at 6 hours after application.

64 Abdominal skin: 76 subjects (14 in the olanexidine gluconate 0.278% group, 12 in the olanexidine gluconate 0.695% group, 13 in the olanexidine gluconate 1.39% group, 13 in the physiological saline group, 12 in the PVI 10% group, and 12 in the CHG 0.5% group)
Inguinal skin: 84 subjects (13 in the olanexidine gluconate 0.278% group, 14 in the olanexidine gluconate 0.695% group, 15 in the olanexidine gluconate 1.39% group, 13 in the physiological saline group, 14 in the PVI 10% group, and 15 in the CHG 0.5% group)

Table 18. Decrease in viable bacterial count from baseline and its difference between active agents and saline

	Olanexidine gluconate 0.278%	Olanexidine gluconate 0.695%	Olanexidine gluconate 1.39%	PVI 10%	CHG 0.5%	Saline
Abdominal skin						
No. of subjects	14	12	13	12	12	13
Baseline	2.159 ± 1.307	2.145 ± 0.260	2.270 ± 1.207	2.208 ± 0.887	2.286 ± 0.536	2.450 ± 0.645
10 min after application	0.981 ± 1.731	0.749 ± 1.636	0.685 ± 1.226	0.688 ± 1.466	0.866 ± 1.526	2.258 ± 1.025
Decrease in viable bacterial count	1.177 ± 1.596	1.396 ± 1.736	1.585 ± 1.785	1.521 ± 1.984	1.420 ± 1.236	0.192 ± 1.282
Difference from saline [95% CI]	0.986 [-0.168, 2.139]	1.204 [-0.051, 2.460]	1.394 [0.136, 2.652]	1.329 [-0.041, 2.700]	1.228 [0.185, 2.272]	
6 hours after application	1.484 ± 1.928	1.104 ± 1.569	0.654 ± 0.668	0.649 ± 1.295	1.113 ± 0.539	2.084 ± 1.158
Decrease in viable bacterial count	0.675 ± 1.701	1.041 ± 1.710	1.616 ± 1.324	1.559 ± 1.858	1.173 ± 0.931	0.366 ± 1.385
Difference from saline [95% CI]	0.309 [-0.926, 1.544]	0.675 [-0.608, 1.958]	1.250 [0.153, 2.347]	1.193 [-0.156, 2.542]	0.807 [-0.178, 1.793]	
Inguinal region						
No. of subjects	13	14	15	14	15	13
Baseline	4.920 ± 1.666	5.114 ± 0.656	5.451 ± 0.695	5.384 ± 1.023	5.025 ± 1.279	4.631 ± 1.121
10 min after application	2.613 ± 0.942	0.961 ± 0.958	0.647 ± 0.782	0.504 ± 0.723	0.747 ± 0.632	3.842 ± 1.267
Decrease in viable bacterial count	2.307 ± 1.989	4.153 ± 1.243	4.804 ± 0.665	4.879 ± 1.294	4.278 ± 1.627	0.788 ± 1.703
Difference from saline [95% CI]	1.518 [0.020, 3.017]	3.364 [2.189, 4.540]	4.016 [2.946, 5.086]	4.091 [2.898, 5.284]	3.490 [2.195, 4.785]	
6 hours after application	2.743 ± 0.935	2.243 ± 1.013	2.202 ± 1.157	0.926 ± 1.438	1.571 ± 0.692	3.744 ± 1.417
Decrease in viable bacterial count	2.177 ± 1.822	2.871 ± 1.056	3.249 ± 1.515	4.457 ± 2.202	3.454 ± 1.510	0.887 ± 1.814
Difference from saline [95% CI]	1.290 [-0.182, 2.762]	1.984 [0.776, 3.191]	2.362 [1.069, 3.655]	3.570 [1.964, 5.177]	2.567 [1.276, 3.858]	

Mean ± standard deviation (Log_{10} CFU/cm^2)

Adverse events, including abnormal laboratory values, were blood creatine phosphokinase (CPK) increased in 1 subject receiving olanexidine gluconate 1.39% to the abdominal skin, diarrhoea in 1 subject receiving olanexidine gluconate 0.695% to the inguinal skin, rash in 1 subject receiving CHG 0.5% to the inguinal skin, and airway inflammation, body temperature increased, lymphocyte count decreased, neutrophil count increased, and white blood cell count increased in 1 subject receiving physiological saline to the abdominal skin. Diarrhoea in the subject receiving olanexidine gluconate 0.695% to the inguinal skin was reported as an adverse drug reaction to the study drug, namely, an adverse event for which a causal relationship to the study drug could not be ruled out.

No deaths, serious adverse events, or adverse events resulting in the discontinuation of study treatment were observed.

4.(iii).A.(2).2) Phase I/II study (5.3.5.1-2: Study 131-202; [[redacted], [redacted] to [redacted], [redacted]])

In Japan, 2 clinical studies in healthy adults (Studies 1 and 2) were conducted at 3 study sites and a related *ex vivo* study at a study site. The target sample size was 294 subjects for the 3 studies combined. Study 1 was a randomized, open-label, parallel-group study in 63 subjects (9 each in the 3 olanexidine gluconate groups, 18 each in the physiological saline and vehicle groups) to assess the safety and pharmacokinetic characteristics of olanexidine gluconate solutions. Study 2 was a randomized open-label, parallel-group study in 216 subjects

(36/group[65]), to assess the decrease in viable bacterial count, dose-effect relationship, and the safety of olanexidine gluconate solutions after single applications to the skin, in comparison with viable bacterial count of normal flora in intact skin. A randomized open-label *ex vivo* study was conducted at a study site as a preparatory study for Study 2 to assess the effect of an inactivating agent on the antiseptic effect of olanexidine gluconate (the inactivating agent was added to skin samples for the measurement of viable bacterial count) (for pharmacokinetic data, see "4.(ii).A.(3) Phase I/II single-dose study").

In Study 1, subjects received a 2-minute application of olanexidine gluconate 1%, 1.5%, or 2% solution, physiological saline, or the vehicle for olanexidine gluconate 2% solution to the skin on the right and left sides of the abdomen (130 cm^2 on each side [260 cm^2 in total]) and the skin on the right and left sides of the inguinal region (30 cm^2 on each side [60 cm^2 in total]).

In the *ex vivo* study, subjects received a 2-minute application of olanexidine gluconate 2% solution, PVI 10% solution, or CHG 0.5% solution to a 49 cm^2 area of the skin on the left side of the abdomen. The olanexidine concentrations on the application site was measured at 10 and 15 minutes after the application.

In Study 2, subjects received a 2-minute application of olanexidine gluconate 1%, 1.5%, or 2% solution, PVI 10% solution, CHG 0.5% solution, or physiological saline to the skin on the right and left sides of the abdomen (49 cm^2 on each side [98 cm^2 in total]) and the skin on the right and left sides of the inguinal region (30 cm^2 on each side [60 cm^2 in total]). Skin samples for viable bacterial count were obtained at baseline and 10 minutes and 6 hours after application.[66]

All 294 randomized subjects (Study 1, n=63; the *ex vivo* study, n=15; Study 2, n=216) received applications of the study drug. The safety analysis set consisted of the 294 subjects: 63 subjects in Study 1 (9, olanexidine gluconate; 18, saline; 18, the vehicle), 15 subjects in the *ex vivo* study (5/group), and 216 subjects in Study 2 (36/group). The full analysis set (FAS) and the efficacy analysis population consisted of 216 subjects in Study 2 (36/group).

Table 19 summarizes the results of primary endpoints: the decrease in viable bacterial count from baseline and its difference between active agents and saline.

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65) In Study 2, subjects who did not meet the following criteria for viable bacterial count at screening were excluded.
- A viable bacterial count of ≥2.0 Log_{10} CFU/cm^2 was observed in at least one sample obtained from the abdominal skin.
- A viable bacterial count of ≥4.0 Log_{10} CFU/cm^2 was observed in at least one sample obtained from the inguinal skin.

66) The skin on the right and left sides of the abdomen and inguinal region was used. Each skin area was divided into 4 compartments: Abdominal skin was divided horizontally and longitudinally into four compartments by two perpendicular lines, while the inguinal skin was divided longitudinally into four compartments. Screening bacteriological testing was performed using samples from the left-most compartment of the left inguinal skin area, the right-most compartment of the right inguinal skin area, the upper-left compartment of the left abdominal skin area, and the upper-right compartment of the right abdominal skin area. The remaining 3 compartments of each skin area were used to obtain samples for viable bacterial count at baseline, 10 minutes, or 6 hours after application.

Table 19. Decrease in viable bacterial count from baseline and its difference between active agents and saline (FAS)

	Olanexidine gluconate 1%	Olanexidine gluconate 1.5%	Olanexidine gluconate 2%	PVI 10%	CHG 0.5%	Saline
Abdominal skin						
No. of skin areas tested	72	72	72	72	72	72
Baseline	2.880 ± 0.546	2.643 ± 0.551	2.793 ± 0.548	2.714 ± 0.595	2.708 ± 0.467[a)]	2.801 ± 0.506
10 min after application	0.620 ± 0.946	0.252 ± 0.623	0.395 ± 0.810	0.252 ± 0.589	0.344 ± 0.514	1.562 ± 0.603
Decrease in viable bacterial count	2.260 ± 0.965	2.391 ± 0.862	2.398 ± 0.834	2.462 ± 0.814	2.359 ± 0.607[a)]	1.239 ± 0.657
Difference from saline [95% CI]	1.021 [0.748, 1.293]	1.152 [0.899, 1.404]	1.159 [0.911, 1.406]	1.222 [0.979, 1.466]	1.119 [0.910, 1.328]	
6 hours after application	1.766 ± 0.896	1.971 ± 1.187	2.050 ± 0.894	1.664 ±1.185	1.896 ± 0.958	2.203 ± 0.861
Decrease in viable bacterial count	1.114 ± 0.875	0.672 ± 1.160	0.743 ± 0.829	1.050 ± 1.304	0.814 ± 0.943[a)]	0.598 ± 0.828
Difference from saline [95% CI]	0.515 [0.235, 0.796]	0.073 [-0.259, 0.406]	0.145 [-0.128, 0.418]	0.451 [0.091, 0.812]	0.215 [-0.078, 0.509]	
Inguinal region						
No. of skin areas tested	72	72	72	72	72	72
Baseline	5.361 ± 1.126	5.221 ± 1.215 [a)]	5.304 ± 1.134	5.168 ± 1.366[a)]	5.480 ± 0.922	5.494 ± 0.929 [a)]
10 min after application	3.053 ± 1.467	2.689 ± 1.752	3.026 ± 1.519 [a)]	1.897 ± 1.440[b)]	2.800 ± 1.264	4.609 ± 0.902
Decrease in viable bacterial count	2.308 ± 1.919	2.557 ± 1.799 [a)]	2.284 ± 1.942 [a)]	3.196 ± 1.953 [c)]	2.680 ± 1.635	0.876 ± 1.247[a)]
Difference from saline [95% CI]	1.432 [0.897, 1.967]	1.681 [1.167, 2.196]	1.408 [0.866, 1.950]	2.321 [1.770, 2.871]	1.805 [1.324, 2.285]	
6 hours after application	3.483 ± 0.733	3.473 ± 0.787	3.415 ± 0.736	3.249 ± 0.798	3.461 ± 0.697	4.409 ± 0.907
Decrease in viable bacterial count	1.878 ± 1.385	1.735 ± 1.333 [a)]	1.890 ± 1.315	1.918 ± 1.534[a)]	2.019 ± 1.138	1.094 ± 1.242 [a)]
Difference from saline [95% CI]	0.783 [0.348, 1.218]	0.640 [0.213, 1.068]	0.795 [0.372, 1.218]	0.824 [0.360, 1.287]	0.925 [0.531, 1.319]	

Mean ± standard deviation (Log_{10} CFU/cm^2)
a) 71 skin areas were evaluated; b) 70 skin areas were evaluated; and c) 69 skin areas were evaluated.

Tables 20 and 21 summarize adverse events, including abnormal laboratory values, observed in Studies 1 and 2, respectively. In the *ex vivo* study, adverse events were observed in 60.0% (3 of 5) of subjects receiving olanexidine gluconate 2% (3 subjects experienced blood LDH decreased, 3 subjects experienced blood CPK decreased, and 1 subject experienced protein total decreased [some subjects experienced more than one adverse event; the same applies hereinafter]), 20.0% (1 of 5) of subjects receiving PVI 10% (1 subject experienced blood LDH decreased and protein total decreased), and 60.0% (3 of 5) of subjects receiving CHG 0.5% (3 subjects experienced blood CPK decreased, 2 subjects experienced blood LDH decreased, and 1 subject experienced protein total decreased). A causal relationship to the study treatment was ruled out in all events.

Table 20. Adverse events in Study 1 (safety analysis set)

Adverse events	Olanexidine gluconate 1%	Olanexidine gluconate 1.5%	Olanexidine gluconate 2%	Saline	Vehicle
No. of subjects	9	9	9	18	18
No. of subjects with adverse events	6 (66.7)	6 (66.7)	6 (66.7)	14 (77.8)	17 (94.4)
Epistaxis	0	0	1 (11.1)	0	0
Diarrhoea	1 (11.1)	0	0	0	1 (5.6)
Dermatitis contact	0	1 (11.1)	0	1 (5.6)	0
Pruritus	0	0	0	0	1 (5.6)
Rash	0	0	0	1 (5.6)	0
Application site dermatitis	0	0	1 (11.1)	0	0
Application site erythema	0	0	1 (11.1)	0	0
ALT increased	1 (11.1)	0	0	0	1 (5.6)
AST increased	1 (11.1)	0	0	0	0
Blood LDH decreased	2 (22.2)	3 (33.3)	6 (66.7)	7 (38.9)	6 (33.3)
Protein total decreased	3 (33.3)	3 (33.3)	1 (11.1)	5 (27.8)	8 (44.4)
Protein total increased	0	1 (11.1)	0	0	0

Adverse events	Olanexidine gluconate 1%	Olanexidine gluconate 1.5%	Olanexidine gluconate 2%	Saline	Vehicle
No. of subjects	9	9	9	18	18
No. of subjects with adverse events	6 (66.7)	6 (66.7)	6 (66.7)	14 (77.8)	17 (94.4)
Blood CPK decreased	3 (33.3)	2 (22.2)	3 (33.3)	5 (27.8)	8 (44.4)

No. of subjects (%)
ALT, alanine aminotransferase; AST aspartate aminotransferase

Table 21. Adverse events in Study 2 (Safety analysis set)

Adverse events	Olanexidine gluconate 1%	Olanexidine gluconate 1.5%	Olanexidine gluconate 2%	Saline	PVI 10%	CHG 0.5%
No. of subjects	36	36	36	36	36	36
No. of subjects with adverse events	20 (55.6)	17 (47.2)	11 (30.6)	22 (61.1)	19 (52.8)	19 (52.8)
Headache	0	0	0	1 (2.8)	0	0
Faeces hard	0	0	0	0	1 (2.8)	0
Dermatitis	0	0	1 (2.8)	0	0	0
ALT decreased	0	0	0	1 (2.8)	0	0
Blood LDH decreased	9 (25.0)	11 (30.6)	3 (8.3)	11 (30.6)	8 (22.2)	11 (30.6)
Differential white blood cell count abnormal	0	0	1 (2.8)	0	0	0
Glucose urine present	0	0	0	0	1 (2.8)	0
Protein total decreased	10 (27.8)	12 (33.3)	3 (8.3)	7 (19.4)	9 (25.0)	5 (13.9)
Blood CPK decreased	8 (22.2)	6 (16.7)	5 (13.9)	11 (30.6)	10 (27.8)	13 (36.1)
Neurologic injury	1 (2.8)	0	0	0	0	0

No. of subjects (%)

In Study 1, 2 adverse drug reactions were observed in the olanexidine gluconate 2% group: mild application site erythema and mild application site dermatitis in 1 subject each. Adverse drug reactions observed in Study 2 were moderate dermatitis in 1 subject in the olanexidine gluconate 2% group and glucose urine present in 1 subject in the PVI 10% group.

No deaths, serious adverse events, or adverse events resulted in the discontinuation of study treatment in any of the studies.

4.(iii).A.(3) Phase III studies

4.(iii).A.(3).1) Phase III studies (5.3.5.1-3, Study 131-302 [[redacted], [redacted] to [redacted], [redacted]])

A randomized, single-blind[67] parallel-group study was conducted in healthy adults in 4 study sites in Japan, to evaluate the efficacy and safety of olanexidine gluconate 1.5% solution versus placebo and CHG 0.5% solution. The target sample size was 600 (240 each in the olanexidine gluconate 1.5% and CHG 0.5% groups, and 120 in the vehicle group).[68]

The subjects received a 2-minute application of olanexidine gluconate 1.5% solution, CHG 0.5% solution, or the vehicle to the skin on the right and left sides of the abdomen (49 cm^2 on each side [98 cm^2 in total]) and

[67] The blind was maintained by not disclosing study drug information to persons involved in the study, except for persons responsible for subject allocation (including persons who actually allocated subjects), persons responsible for the control/accountability of study drug, and persons who applied the study drug to the skin of subjects (including persons who assisted the persons who applied the study drug).

[68] Individuals who satisfied either of the following criteria for viable bacterial count at screening were excluded from the study:
<2.0 Log_{10} CFU/cm^2 in both skin areas (right and left) of the abdomen; or
<4.0 Log_{10} CFU/cm^2 in both skin areas (right and left) of the inguinal region.

the skin on the right and left sides of the inguinal region (30 cm^2 on each side [60 cm^2 in total]). The skin was then allowed to dry without dressing. Skin samples for bacteriological testing were obtained at baseline and 10 minutes and 6 hours after application, to determine the viable bacterial count in each sample.[66]

Among the 600 randomized subjects, 594 subjects who received the study drug (237 in the olanexidine gluconate 1.5% group, 237 subjects in the CHG 0.5% group, and 120 subjects in the vehicle group) were included in the safety analysis set, and 592 subjects (237 in the olanexidine gluconate 1.5% group, 236 subjects in the CHG 0.5% group, and 119 subjects in the vehicle group) were included in the FAS, which was also the analysis set for efficacy evaluation.

Table 22 summarizes the viable bacterial count at baseline and 10 minutes and 6 hours after application of olanexidine gluconate 1.5% solution, CHG 0.5% solution, or the vehicle to the abdominal and inguinal skin. The viable bacterial count at 10 minutes after application to the abdominal and inguinal skin (the primary endpoint) differed significantly between the vehicle and olanexidine gluconate 1.5% groups ($P < 0.001$; In a mixed effects model using treatment group, skin area, and skin compartment as the fixed effects, and subjects as the random effect). This result demonstrated the superiority of olanexidine gluconate 1.5% over the vehicle. The lower limit of the 95% confidence interval of the difference in viable bacterial count at 10 minutes after application between the CHG 0.5% and olanexidine gluconate 1.5% groups, compared by the least square mean (0.118 Log_{10} CFU/cm^2 for the abdominal skin and -0.159 Log_{10} CFU/cm^2 for the inguinal skin) was lower than the predetermined non-inferiority margin (-0.344 for the abdominal skin and -0.482 for the inguinal skin).[69] Thus, the non-inferiority of olanexidine gluconate 1.5% solution over CHG 0.5% was demonstrated.

[69] On the basis of the results of the phase I/II study (Study 131-202), the non-inferiority margin was defined as follows:
Abdominal skin: The efficacy of CHG 0.5% solution in this non-inferiority study (M1) was defined at 1.033 log_{10} CFU/cm^2, which is the lower limit of the 95% confidence interval of the difference in mean viable bacterial count between CHG 0.5% solution and saline in the phase I/II study (Study 131-202). Assuming that non-inferiority of olanexidine gluconate 1.5% solution over CHG 0.5% solution may be demonstrated with two-thirds of M1, the non-inferiority margin for olanexidine gluconate 1.5% solution (M2) was set at 0.344 ([1 - 2/3] × 1.033 = 0.344).
Inguinal skin: The efficacy of CHG 0.5% solution in this non-inferiority study (M1) was defined at 1.447 log_{10} CFU/cm^2, which is the lower limit of the 95% confidence interval of the difference in mean viable bacterial count between CHG 0.5% solution and saline in the phase I/II study (Study 131-202). Assuming that non-inferiority of olanexidine gluconate 1.5% solution over CHG 0.5% solution may be demonstrated with-two thirds of M1, the non-inferiority margin for olanexidine gluconate 1.5% solution (M2) was set at 0.482 ([1 - 2/3] × 1.447 = 0.482).

Table 22. Viable bacterial counts in the abdominal and inguinal skin areas at each time point (FAS)

	Olanexidine gluconate 1.5%	CHG 0.5%	Vehicle
Abdominal skin			
Baseline	2.799 ± 0.514 (474)	2.748 ± 0.543 (471)	2.748 ± 0.576 (238)
10 min after application	0.285 ± 0.728 (474)	0.521 ± 0.803 (471)	1.528 ± 0.780 (238)
6 hours after application	2.048 ± 0.922 (474)	1.992 ± 0.961 (471)	2.159 ± 0.762 (238)
Difference from vehicle at 10 minutes after application [95% CI] a)	1.243 [1.100, 1.386] $P < 0.001$		
Difference from CHG 0.5% at 10 minutes after application [95% CI] a)	0.235 [0.118, 0.353]		
Inguinal region			
Baseline	5.211 ± 1.128 (471)	5.299 ± 1.102 (472)	5.159 ± 1.291 (236)
10 min after application	2.811 ± 1.450 (472)	2.826 ± 1.360 (470)	4.504 ± 0.993 (237)
6 hours after application	3.326 ± 0.784 (473)	3.342 ± 0.766 (470)	4.345 ± 0.832 (238)
Difference from vehicle at 10 minutes after application [95% CI] a)	1.706 [1.505, 1.907] $P < 0.001$		
Difference from CHG 0.5% at 10 minutes after application [95% CI] a)	0.016 [-0.159, 0.191]		

Mean ± standard deviation (Log_{10} CFU/cm^2) (No. of skin areas tested)

a) A mixed effects model using treatment group, skin area, and skin compartment as the fixed effects and subjects as the random effect

Adverse events, including abnormal laboratory values, were observed in 47 of 237 subjects (19.8%) in the olanexidine gluconate group, 44 of 237 subjects (18.6%) in the CHG 0.5% group, and 15 of 120 subjects (12.5%) in the vehicle group. Table 23 summarizes adverse events observed in ≥ 2 subjects in at least one group.

Table 23. Adverse events developing in ≥ 2 subjects in at least one group (Safety analysis set)

Adverse events	Olanexidine gluconate 1.5%	CHG 0.5%	Vehicle
No. of subjects	237	237	120
No. of subjects with adverse events	47 (19.8)	44 (18.6)	15 (12.5)
Diarrhoea	0	2 (0.8)	0
Application site erythema	5 (2.1)	2 (0.8)	1 (0.8)
Protein total decreased	40 (16.9)	33 (13.9)	14 (11.7)

No. of subjects (%)

The incidence of adverse drug reactions was 1.3% (3 of 237 subjects) in the olanexidine gluconate 1.5% group, 0.8% (2 of 237 subjects) in the CHG 0.5% group, and 0.8% (1 of 120 subjects) in the vehicle group. All adverse drug reactions were slight application site erythema, with the outcome of "recovered/resolved."

No deaths, serious adverse events, or adverse events resulted in the discontinuation of study treatment.

4.(iii).A.(3).2) Phase III study (5.3.5.1-4, Study 131-301 [[redacted], [redacted] to [redacted], [redacted]])

A randomized, open-label, parallel-group study was conducted in patients scheduled for laparoscopic surgery[70] for the treatment of gastrointestinal disorder at 16 study sites in Japan, to assess the safety and pharmacokinetics of olanexidine gluconate 1.5% versus PVI 10%. The target sample size was 110 (56 in the olanexidine gluconate 1.5% group and 54 in the PVI 10% group) (for pharmacokinetic data, see "4.(ii).A.(4) Phase III study").

[70] Patients scheduled for gastrectomy, colectomy, or rectal resection under laparoscopic surgery (without perineal procedures).

The patients received a single application of olanexidine gluconate 1.5% solution or PVI 10% solution to the skin at the surgical site immediately before surgery. The skin was then allowed to dry without dressing.[71] The patients were monitored and tested during the period between application and 7 days after surgery.

Among the 110 randomized patients, 106 patients who received the study drug (52 patients receiving olanexidine gluconate 1.5% and 54 patients receiving PVI 10%) were included in the safety analysis set.

Adverse events, including abnormal laboratory values, were observed in 36 of 52 patients (69.2%) in the olanexidine gluconate 1.5% group and 38 of 54 patients (70.4%) in the PVI 10% group. Table 24 summarizes adverse events observed in ≥ 2 subjects in at least one group.

Table 24. Adverse events developing in ≥ 2 subjects in at least one group (Safety analysis set)

Adverse events	Olanexidine gluconate 1.5%	PVI 10%
No. of subjects	52	54
No. of subjects with adverse events	36 (69.2)	38 (70.4)
Application site erythema	14 (26.9)	11 (20.4)
Blood pressure decreased	5 (9.6)	5 (9.3)
Erythema	3 (5.8)	2 (3.7)
Wound complication	3 (5.8)	0
Blood CPK increased	2 (3.8)	4 (7.4)
Nausea	2 (3.8)	3 (5.6)
Blood pressure increased	2 (3.8)	2 (3.7)
Pain	2 (3.8)	2 (3.7)
Hyponatraemia	2 (3.8)	0
Delirium	2 (3.8)	0
Insomnia	2 (3.8)	1 (1.9)
Constipation	2 (3.8)	0
Vomiting	2 (3.8)	1 (1.9)
Application site haematoma	1 (1.9)	7(13.0)
Blister	1 (1.9)	2 (3.7)
Ileus	1 (1.9)	2 (3.7)
Musculoskeletal pain	1 (1.9)	2 (3.7)
Suture rupture	0	3 (5.6)

No. of subjects (%)

Adverse drug reactions were observed in 5.8% (3 of 52) of patients in the olanexidine gluconate 1.5% group (application site dermatitis, application site erythema, and application site pruritus in 1 patient each) and 7.4% (4 of 54) of patients in the PVI 10% group (application site erythema in 4 patients).

No deaths occurred during the study. During the follow-up period,[72] serious adverse events were observed in 2 patients in the olanexidine gluconate group (peritonitis and postoperative wound infection in 1 patient each), 5 patients in the PVI 10% group (suture rupture in 3 patients and inflammation and ileus in 1 patient each). Serious adverse events occurred after the follow-up period in 2 patients in the olanexidine gluconate group

[71] After the application of the study drug, the skin was allowed to dry without dressing before it was covered by a gown or drape. After surgery, the incision was covered with an appropriate film dressing. The dressing was changed whenever necessary depending on the observed condition of the incision on day 1 or 2 after surgery. No dressing was used from day 3 after surgery onward.

[72] The follow-up period was from the informed consent to the completion of observation and examinations on day 7 or 8 after surgery.

(ileus and gastric dilatation in 1 patient each), and 3 patients in the PVI 10% group (postoperative wound infection, ascites, and intestinal obstruction in 1 patient each). A causal relationship to study treatment was ruled out for all events. The outcome was reported as "recovered/resolved" or "recovering/resolving" for all events.

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

4.(iii).B.(1) Efficacy

PMDA reviewed the submitted data, as described below, and concluded that the data demonstrated the efficacy of olanexidine gluconate 1.5% solution as an antiseptic for intact skin at the surgical site.

This conclusion will be discussed at the Expert Discussion.

4.(iii).B.(1).1) Efficacy evaluation of olanexidine gluconate 1.5% solution

PMDA's view:

The clinical efficacy of antiseptic agents can be assessed based on the change in viable bacterial count on the skin treated with the agents, because antiseptic agents are used to reduce normal skin flora and eliminate bacteria and pathogens on the skin that may cause surgical wound infection.[73] Accordingly, the efficacy of olanexidine gluconate 1.5% solution in preoperative skin preparation at the surgical site in Japanese patients scheduled for surgery can be assessed using the data of clinical studies in healthy adults.

According to the "Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for Health-Care Antiseptic Drug Products, 21 CFR Part 333" (hereinafter referred to as the "TFM"), the clinical assessment of antiseptics for the preoperative skin preparation at the surgical site can be performed based on the decrease in viable bacterial count of normal flora on the skin of healthy adults in the United States.[74]

4.(iii).B.(1).2) Efficacy

PMDA asked the applicant to justify the reasons the study drug was applied to the skin for 2 minutes, and skin samples for bacteriological testing were obtained at 10 minutes after application in the phase II study (Study 131-201), phase I/II study (Study 131-202), and phase III study (Study 131-302), all conducted in Japan.

The applicant's explanation:

The study drug was applied for 2 minutes as the skin should be in contact with an antiseptic agent for a sufficient length of time to ensure the elimination of microorganisms on the skin. The Practical Guidelines for

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[73] Sebben JE. *J Am Acad Dermatol*. 9: 1983;759-765.

[74] Federal Register. Topical antimicrobial drug products for over-the-counter human use; tentative final monograph for health-care antiseptic drug products. June 17, 1994;59(116):31402-31452.

Surgical Treatment, proposed by the Japanese Association for Operative Medicine,[75] recommend that "the skin be exposed to an antiseptic agent for 2 to 3 minutes to ensure its efficacy." The 2-minute application time is thus appropriate.

The *in vitro* study of the bactericidal activity of olanexidine gluconate 1.5% solution revealed that olanexidine gluconate 1.5% exerted a bactericidal activity against various Gram-positive and Gram-negative bacteria, with a similar bactericidal effect to that of CHG 0.5% and PVI 10%, after a 30-second to 3-minute application (see "3.(i).A.(1) Primary pharmacodynamics"); this suggests that skin should be exposed to antiseptic agents for several minutes to ensure their antimicrobial activity. No Japanese guidelines for the assessment of antiseptic agents were available when the protocol for the phase III study (Study 131-302) was developed. However, the TFM,[74] which is used in the clinical assessment of new antiseptic agents in the United States, recommends that the efficacy of an antiseptic agent be assessed based on the viable bacterial count in skin samples obtained at 10 minutes after application.[76] Accordingly, skin samples were collected at 10 minutes after application of the study drug.

In the phase III study (Study 131-301), the time between the completion of study drug application and the initiation of surgery was 12.2 ± 5.1 minutes (mean ± standard deviation). Assessing the antiseptic effect of an agent at 10 minutes after the completion of application is thus appropriate.

The applicant’s explanation on the concentration of olanexidine gluconate solution used in the phase III study (Study 131-302):
Table 25 shows the decrease in viable bacterial count at 10 minutes after study drug application and its difference between the olanexidine gluconate groups and the saline group in Study 2 of the Phase I/II study (Study 131-102). The difference from saline in viable bacterial counts in the abdominal and inguinal skin areas was similar for all concentrations of olanexidine gluconate tested. Thus an olanexidine gluconate solution at a high concentration should be used to ensure an appropriate antiseptic action, as long as there are no concerns regarding safety. Since a patient receiving olanexidine gluconate 2% solution experienced a moderate or severe adverse drug reaction (dermatitis), olanexidine gluconate 1.5% solution was selected for the phase III study (Study 131-302).

[75] Harihara Y, *The Journal of the Japanese Association for Operating Room Technology*. 2013;34 (Suppl.):S58-S70.

[76] The TFM recommends that the efficacy of over-the-counter (OTC) health care antiseptic agents for the preoperative skin preparation at the surgical site be assessed based on the reduction in normal skin flora from baseline to a time point within 10 minutes after application (before application).

Table 25. Decrease in viable bacterial count from baseline to 10 minutes after application and its difference between olanexidine gluconate and saline (FAS) (same data as Table 19)

	Olanexidine gluconate 1%	Olanexidine gluconate 1.5%	Olanexidine gluconate 2%	Saline
Abdominal skin				
No. of skin areas tested	72	72	72	72
Baseline	2.880 ± 0.546	2.643 ± 0.551	2.793 ± 0.548	2.801 ± 0.506
10 min after application	0.620 ± 0.946	0.252 ± 0.623	0.395 ± 0.810	1.562 ± 0.603
Decrease in viable bacterial count	2.260 ± 0.965	2.391 ± 0.862	2.398 ± 0.834	1.239 ± 0.657
Difference from saline [95% CI]	1.021 [0.748,1.293]	1.152 [0.899,1.404]	1.159 [0.911,1.406]	
Inguinal region				
No. of skin areas tested	72	72	72	72
Baseline	5.361 ± 1.126	5.221 ± 1.215 a)	5.304 ± 1.134	5.494 ± 0.929 a)
10 min after application	3.053 ± 1.467	2.689 ± 1.752	3.026 ± 1.519 a)	4.609 ± 0.902
Decrease in viable bacterial count	2.308 ± 1.919	2.557 ± 1.799 a)	2.284 ± 1.942 a)	0.876 ± 1.247 a)
Difference from saline [95% CI]	1.432 [0.897,1.967]	1.681 [1.167,2.196]	1.408 [0.866,1.950]	

Mean ± standard deviation (Log_{10} CFU/cm^2)
a) No. of skin areas tested: 71

The applicant's explanation on the results of efficacy evaluation:

As shown in Table 22, the phase III study (Study 131-302) revealed statistically significant differences in the viable bacterial count at 10 minutes after application between olanexidine gluconate 1.5% solution and the vehicle control ($P < 0.001$; In a mixed effects model using treatment group, skin area, and skin compartment as the fixed effects and patients as the random effect). This result showed the superiority of olanexidine gluconate 1.5% over the vehicle both in the abdominal and inguinal skin. Table 22 shows data regarding the efficacy of olanexidine gluconate 1.5% versus CHG 0.5%, as evaluated by the viable bacterial count at 10 minutes after application. The difference between olanexidine gluconate 1.5% and CHG 0.5% in the least square mean (95% confidence interval) of viable bacterial count at 10 minutes after application was 0.235 (0.118, 0.353) Log_{10} CFU/cm^2 in the abdominal skin and 0.016 (-0.159, 0.191) Log_{10} CFU/cm^2 in the inguinal skin; their lower limits of 95% CI were higher than the predefined margin of inferiority (-0.344 Log_{10} CFU/cm^2 in the abdominal skin and -0.482 Log_{10} CFU/cm^2 in the inguinal skin).[69] The non-inferiority of the olanexidine gluconate over CHG was thus demonstrated.

PMDA's view:

The applicant decided to use the 2-minute application time for the clinical studies of olanexidine gluconate solutions according to the recommendation of the Practical Guidelines for Surgical Treatment (the Journal of Japanese Association for Operating Room Technology),[75] which states that an approximately 2- to 3-minute exposure to an antiseptic agent is necessary to ensure sufficient antiseptic effects. The applicant also decided to collect skin samples for bacteriological testing at 10 minutes after study drug application, based on *in vitro* data (regarding the onset of effects of antiseptic agents) and the guidelines in the United States on the efficacy assessment of antiseptics for the preoperative skin preparation at the surgical site. These decisions by the applicant are acceptable.

The phase III study (Study 131-302) demonstrated the superiority of olanexidine gluconate 1.5% over the

vehicle, and showed almost no difference between olanexidine gluconate 1.5% and CHG 0.5% in the viable bacterial counts on the abdominal and inguinal skin at 10 minutes after application. Olanexidine gluconate 1.5% solution is thus expected to be effective.

4.(iii).B.(2) Safety

PMDA reviewed the safety of olanexidine gluconate 1.5% solution, as described in 4.(iii).B.(2).1) to 4), and concluded that olanexidine gluconate 1.5% solution was tolerable. The clinical study in patients scheduled for surgery (Study 131-301) enrolled only patients scheduled for laparoscopic surgery for the treatment of gastrointestinal disorder. However, olanexidine gluconate 1.5% solution is expected to be used for the preoperative skin preparation at the surgical site in patients undergoing other types of surgical procedures as well. Therefore the applicant should collect data on the safety of olanexidine gluconate 1.5% solution in patients undergoing surgical procedures other than laparoscopic surgery in the post-marketing surveillance.

The above conclusion of PMDA will be discussed at the Expert Discussion.

4.(iii).B.(2).1) Summary of safety

The applicant's explanation on the safety of olanexidine gluconate solution applied to the skin in the clinical studies:

In a pooled analysis of the data from healthy adults enrolled in the phase II (Study 131-201), phase I/II (Study 131-202), phase I (Study 131-104), and phase III (Study 131-302) studies, the incidence of adverse events was 26.4% (131 of 497 subjects) in the olanexidine gluconate 0.278% to 2% groups, 22.0% (67 of 305 subjects) in the CHG 0.5% group, 29.9% (20 of 67 subjects) in the PVI 10% group, 22.8% (36 of 158 subjects) in the vehicle group, and 46.3% (37 of 80 subjects) in the saline group. The incidence of adverse drug reactions at application site was 0.8% (4 of 497 subjects) for application site erythema and 0.2% (1 of 497 subjects) for application site dermatitis. These events were all mild in severity and reversible.

In the phase III study (Study 131-301) in patients scheduled for laparoscopic surgery for the treatment of gastrointestinal disorder, adverse events developed in 69.2% (36 of 52) of patients in the olanexidine gluconate group and 70.4% (38 of 54) of patients in the PVI 10% group. Adverse drug reactions developed in 5.8% (3 of 52) of patients in the olanexidine gluconate 1.5% group (application site dermatitis, application site erythema, and application site pruritus in 1 patient each) and 7.4% (4 of 54) of patients in the PVI 10% group (application site erythema in 4 patients). The profiles of adverse events and adverse drug reactions did not differ between the olanexidine gluconate 1.5% and PVI 10% groups. Adverse drug reactions observed in the olanexidine gluconate 1.5% group were moderate in severity and reversible.

These findings suggest that there are no specific problems with the safety of olanexidine gluconate 1.5% solution.

PMDA's view:

The incidence and profile of adverse events observed in healthy adults receiving olanexidine gluconate solutions did not differ substantially from those receiving CHG 0.5%, PVI 10%, the vehicle, or physiological saline. Adverse events developing at the application site were mild in severity, and did not cause any particular problems. There are no major concerns on the safety of the preoperative application of olanexidine gluconate 1.5% solution in patients undergoing laparoscopic surgery. The sections below discuss the safety of olanexidine gluconate 1.5% solution applied to damaged skin and the possible systemic effect of olanexidine gluconate distributed into blood. Olanexidine gluconate 1.5% solution is to be applied to the skin, and is not readily absorbed through the skin. However, since decreased total protein and other abnormal laboratory values (increases in blood ALT, blood AST, blood LDH, and blood CPK) developed in subjects participating in the clinical studies, healthcare professionals must be appropriately informed of the occurrence of these adverse events.

4.(iii).B.(2).2) Safety of the application of olanexidine gluconate 1.5% solution to damaged skin

PMDA requested the applicant to explain the safety of the application of olanexidine gluconate 1.5% solution to damaged skin.

The applicant's explanation:

^{14}C-labeled olanexidine gluconate 0.695% solution was applied to abraded skin on the back of male rats for up to 24 hours with occlusive dressing. The C_{max} and $AUC_{0\text{-}24h}$ of serum olanexidine concentration in the rats (C_{max}, 11.9 ng/mL; $AUC_{0\text{-}24h}$, 142 ng·h/mL) were 6.8- and 13.7-fold higher, respectively, than those on intact skin (C_{max}, 1.76 ng/mL; $AUC_{0\text{-}24h}$, 10.4 ng·h/mL) (Table 15). The C_{max} (11.9 ng/mL) was 1.2- to 1.4-fold higher than C_{max} (9.65 to 8.30 ng/mL from Day 1 to the last dose) in male rats receiving 4-week repeated subcutaneous injections of 0.278 mg/kg (NOAEL) of olanexidine gluconate. The $AUC_{0\text{-}24h}$ (142 ng·h/mL) was 1.9- to 2.9-fold higher than $AUC_{0\text{-}24h}$ (48.3 to 75.5 ng·h/mL from Day 1 to the last dose) in male rats receiving 4-week repeated subcutaneous injections of 0.278 mg/kg (NOAEL) of olanexidine gluconate (Table 13). These findings suggest that the application of olanexidine gluconate solution to damaged human skin may increase safety risks, because serum olanexidine concentration will become higher after application to damaged skin than after application to intact skin (see "3.(ii).A.(1) Absorption" and "3.(iii).A.(6) Other toxicity studies"). In the skin irritation study where olanexidine gluconate solutions were applied to intact skin and skin without stratum corneum with no dressing on the back of rabbits, olanexidine gluconate ≤0.695% solutions did not cause skin irritation in either intact skin or skin without stratum corneum, while olanexidine gluconate 6.95% solution caused stronger irritation in skin without stratum corneum than in intact skin.[77] However, olanexidine gluconate is not highly irritating even at the concentration of 6.95% (see "3.(iii).A.(5) Local tolerance").

[77] When skin reactions were scored according to Draize scoring criteria, the highest mean scores in intact skin and skin without stratum corneum were 1.17 and 1.83, respectively.

The safety of olanexidine gluconate 1.5% solution applied to wound sites in humans has not been established because of the absence of its clinical experience. CHG, a substance with a chemical structure similar to olanexidine gluconate, has been reported to cause shock when applied to a wound site; the use of CHG to the brain, spinal cord, and ear (inner, middle, and outer ear)[78] and mucosal surface (e.g., vaginal mucosa, urinary bladder mucosa, and oral mucosa)[79] is contraindicated.[80] A study of cytotoxicity of olanexidine gluconate on human-derived cells revealed that the cytotoxicity of olanexidine gluconate is similar to that of CHG (see "3.(i).A.(2) Secondary pharmacodynamics"), but olanexidine gluconate may cause stronger tissue damage at the application site than CHG, considering the concentrations of olanexidine gluconate and CHG solutions to be used in the clinical setting.[81] The possibility that olanexidine gluconate may induce anaphylaxis symptoms cannot be ruled out because such symptoms have been reported with CHG. PVI 10% solution and CHG 0.5% solution, antiseptics marketed in Japan, are indicated not only for "preoperative skin preparation at the surgical site," but also for "disinfection of mucous membrane" and "disinfection of wound sites" etc. Therefore healthcare professionals must be advised not to use olanexidine gluconate solution for purposes other than "preoperative skin preparation at the surgical site." The Practical Guidelines for Surgical Treatment, proposed by the Japanese Association for Operative Medicine,[75] describe that "Sutured surgical wounds should be covered with sterilized dressing for 24 to 48 hours after surgery. No dressing is necessary thereafter. Disinfection is usually not necessary for sutured surgical wounds." The olanexidine gluconate 1.5% solution should not be used after surgery or on wound sites.

On the basis of the above findings and the descriptions in the package insert for currently available antiseptics, the applicant plans to conduct the following measures: The package insert for olanexidine gluconate 1.5% solution will include a statement that prohibits the application of the solution to damaged skin or mucosa; and information related to the package insert will be provided to healthcare professionals in order to educate them and ensure the proper use of olanexidine gluconate 1.5% solution in the post-marketing settings.

PMDA' view on the safety of olanexidine gluconate 1.5% solution applied to damaged skin:
No particular safety problems associated with olanexidine gluconate 1.5% solution were identified in the clinical study (Study 131-301) in patients scheduled for laparoscopic surgery for the treatment of gastrointestinal disorder. However, the number of patients enrolled in the study was limited, and findings from non-clinical studies indicate that serum olanexidine concentration increases when olanexidine gluconate is applied to damaged skin and olanexidine gluconate is more irritating when it is applied to skin without stratum corneum. CHG, a substance with an antiseptic characteristics similar to olanexidine gluconate, has been

[78] When the auditory nerve and the central nervous system are directly exposed to CHG, hearing loss or nervous system disorders may develop.

[79] Chlorhexidine preparations have been reported to induce symptoms of shock (early symptoms include nausea, discomfort, cold sweat, dizziness, chest distress, dyspnea, and rash, etc.).

[80] Takahashi A, et al. *Journal of healthcare-associated infection*. 2008;1:35-38.

[81] Olanexidine gluconate 1.5% solution and CHG 0.1% to 0.5% solutions are used for the preoperative skin preparation at the surgical site. CHG 0.05% solution is used for disinfection of the wound site.

reported to cause shock when applied to a wound site, and the use of CHG to the brain, spinal cord, or ear (inner ear, middle ear, and outer ear) or mucosal surface (e.g., vaginal mucosa, urinary bladder mucosa, and oral mucosa) is contraindicated. These findings indicate that olanexidine gluconate 1.5% solution may cause similar safety problems when applied to these tissues and organs. Accordingly, healthcare professionals should be instructed not to apply olanexidine gluconate 1.5% solution to surgical wounds or damaged skin after surgery. As currently available antiseptic agents (PVI and CHG) are allowed to be applied to wound sites, the applicant should provide sufficient information through the package insert and other materials to instruct healthcare professionals not to apply olanexidine gluconate 1.5% solution to wounds or damaged skin, in order to ensure the proper use of olanexidine gluconate 1.5% solution. Cautions should be also indicated on the label on the bottle and applicators of Olanedine. The applicant should continue to collect information regarding the safety of olanexidine gluconate 1.5% solution through the post-marketing surveillance and other studies. If any new concerns arise with the use of olanexidine gluconate 1.5% solution, healthcare professionals should be informed of the concerns promptly.

4.(iii).B.(2).3) Systemic effect of olanexidine gluconate distributed into blood

The applicant's explanation on the possible systemic effect of olanexidine gluconate distributed from the site of application to the blood:

Olanexidine and its metabolites were detected in the serum of many patients in the phase III study (Study 131-301). Olanexidine gluconate solution applied on the skin was probably transferred into the body through skin incisions and other surgical procedures, resulting in the detection of olanexidine in the serum. The serum concentrations of olanexidine and its metabolites differed among patients but were not related to the incidence of adverse events. A causal relationship to olanexidine gluconate 1.5% solution was ruled out in all adverse events developing in subjects with a relatively high serum olanexidine concentration (C_{max} ≥0.300 ng/mL). Systemic exposure to olanexidine and its metabolites is thus unlikely to affect the safety of olanexidine gluconate 1.5% solution in the clinical setting (see the subsection "Systemic exposure to olanexidine and its metabolites" in Section "4.(ii).B Outline of the review by PMDA").

The safety of olanexidine gluconate 1.5% solution was not assessed in patients undergoing surgical procedures other than laparoscopic surgery. However, the results of a non-clinical study in minipigs suggest that the type of surgical procedures or incision length does not affect serum olanexidine concentration (see Section "3.(ii).A.(6) Others").

These findings suggest that the distribution of olanexidine gluconate into the body through laparoscopic surgery or other surgical procedures is unlikely to cause safety concerns. However, the safety of olanexidine gluconate 1.5% solution in patients undergoing various surgical procedures, including laparoscopic surgery, will be assessed in the postmarketing surveillance.

PMDA's view:

As mentioned in the subsection "Systemic exposure to olanexidine and its metabolites" in Section "4.(ii).B Outline of the review by PMDA," the systemic exposure to olanexidine gluconate may be affected by various factors such as the amount of the drug applied, the time from the completion of drug application to the start of surgery, duration of surgery, the amount of bleeding during surgery, and type of surgery (single incision laparoscopic surgery vs. other procedures). The amount of olanexidine gluconate distributed into the body may thus differ by site or type of surgery. However, since no significant systemic adverse events occurred in the clinical studies, the distribution of olanexidine gluconate into the body during surgical procedures other than laparoscopic surgery is unlikely to cause significant safety problems, as long as the proper use of olanexidine gluconate 1.5% solution is ensured by instructing healthcare professionals not to apply the drug to damaged skin. Only limited data are available regarding the safety of olanexidine gluconate 1.5% solution in patients undergoing laparoscopic surgery, and no safety data are available in patients undergoing other types of surgery. During the postmarketing surveillance, safety information should thus be collected from a variety of patients and provided to healthcare professionals in an appropriate manner.

4.(iii).B.(3) Indications and clinical positioning

The proposed indication for olanexidine gluconate 1.5% solution is preoperative skin preparation at the surgical site. PMDA asked the applicant to list major causes of surgical site infection (SSI) and provide rationale for the proposed indication.

The applicant’s explanation:

In the Japan Nosocomial Infections Surveillance,[82] the incidence and the five most common causes of SSI are summarized by type of surgery: "gastrointestinal surgery," "cardiovascular surgery," "orthopedic surgery," and "general surgery, neurosurgery, gynecologic surgery, urological surgery, and ear, nose, and throat (ENT) surgery." Table 26 summarizes the five most common causes of SSI by surgical procedure.[83]

[82] Japan Nosocomial Infections Surveillance Surgical Site Infection Division of the Japan Nosocomial Infections Surveillance. The Ministry of Health, Labor and Welfare. Nosocomial infection surveillance annual report in 2013. http://www.nih-janis.jp/report/ssi.html (Accessed in February 2015)

[83] Methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant enterococci (VRE) were tabulated separately from drug-susceptible strains.

Table 26. The five most common causes of SSI by surgical procedure

Organ system	Surgical procedures	Five most common causes of SSI by surgical procedure
Gastrointestinal surgery	Esophageal surgery, hepatobiliary/pancreatic surgery, small intestine surgery, large intestine surgery, gastric surgery, appendix surgery, gallbladder surgery, spleen surgery, hernia surgery, and liver transplantation	*E. faecalis* *P. aeruginosa* *E. cloacae* *E. coli* *Bacteroides fragilis* group
Cardiovascular surgery	Thoracic aortic surgery, coronary artery bypass grafting with chest incision only, peripheral vascular bypass surgery, abdominal aortic repair, cardiac surgery, abdominal aortic endovascular surgery, carotid endarterectomy, shunt surgery for dialysis, pacemaker surgery, chest aortic endovascular surgery, varicose vein surgery, coronary artery bypass grafting with incisions in the chest and graft site, and heart transplantation.	MRSA *S. aureus* *S. epidermidis* *Corynebacterium* spp. CNS *P. aeruginosa*
Orthopedic surgery	Limb amputation, spinal fusion surgery, rachiotomy, open reduction of fractures, hip arthroplasty, knee arthroplasty, and repeat spinal fusion surgery.	MRSA *S. aureus* *S. epidermidis* CNS *E. cloacae*
General surgery, neurosurgery, gynecologic surgery, urological surgery, ENT surgery	Abdominal surgery, neck surgery, abdominal hysterotomy, renal transplantation, renal surgery, mastectomy, craniotomy, ventricular shunt, thoracic surgery, prostatic surgery, ovarian surgery, caesarean section, vaginal hysterectomy, thyroid surgery, and parathyroid surgery	MRSA *S. aureus* *P. aeruginosa* *E. faecalis* *S. epidermidis*

As preoperative skin preparation at the surgical site is performed to reduce normal flora and eliminate bacteria and pathogens on the skin which may cause surgical wound infection,[73] olanexidine gluconate 1.5% solution must exert bactericidal activity against bacteria on the surface of the skin. Olanexidine gluconate is expected to be effective on different surgical sites, as long as the surgical sites possess similar types of bacteria and olanexidine gluconate has bactericidal activity against the bacteria. Normal skin flora consists of 6 species: Gram-positive cocci (*Staphylococcus* sp. and *Micrococcus* sp.), Gram-positive rods (*Corynebacterium* sp., *Propionibacterium* sp., and *Brevibacterium* sp.), and Gram-negative rods (*Acinetobacter* sp.). The most common causes of SSI are listed in Table 26.[84] Olanexidine gluconate exerts its bactericidal activity against all of these bacteria (see "3.(i).B.(1) Mechanism of action and bactericidal activity of olanexidine gluconate"). In the phase III study (Study 131-302), olanexidine gluconate 1.5% was shown to be superior to the vehicle and non-inferior to CHG 0.5% in the viable bacterial count at 10 minutes after application to abdominal and inguinal skin.

On the basis of the above findings, the proposed wording for the indication, "preoperative skin preparation at the surgical site" (with no mention of specific type of bacteria covered, as with the indication for other available antiseptics), is considered appropriate. Olanexidine gluconate 1.5% solution is expected to exert a bactericidal effect against clinically significant bacteria, such as MRSA, VRE, *P. aeruginosa*, and *Serratia* sp., and antiseptic-resistant bacteria such as *B. cepacia*. Therefore, providing healthcare professionals and patients with olanexidine gluconate 1.5% solution as a skin antiseptic for the prophylaxis of postoperative wound infection is thought to be meaningful.

[84] Wilson M. *Microbial inhabitants of humans*. 2005;65-76, Miyaji Y, Naganuma M. *Dermatology for researchers of cosmetics and drugs for external use*. 2005;40-44.

PMDA’s view:

The proposed indication, "preoperative skin preparation at the surgical site," is acceptable on the basis of the discussion presented in the sections "4.(iii).B.(1) Efficacy" and "4.(iii).B.(2) Safety." Olanexidine gluconate 1.5% solution can be an option for preoperative skin preparation at the surgical site. However, the difference in site or type of surgery may affect the absorption of olanexidine gluconate, and no data are available on the safety of olanexidine gluconate 1.5% solution in patients who received the drug prior to surgical procedures other than laparoscopic surgery. Thus, safety information should be collected from a variety of patients and provided appropriately to healthcare professionals during the postmarketing surveillance (see "4.(iii).B.(2) Safety" and "4.(iii).B.(2).3) Systemic effect of olanexidine gluconate distributed into blood").

The above conclusion of PMDA will be discussed at the Expert Discussion.

4.(iii).B.(4) Dosage and administration

The proposed dosage and administration is "Olanedine is applied to the skin," while in the phase III study (Study 131-301) olanexidine gluconate 1.5% solution was applied once to the skin and dried without dressing before surgery. PMDA asked the applicant to explain the length of time required to dry the skin and the length of time required between application of the solution and the beginning of surgery or procedure.

The applicant’s explanation:

The Practical Guidelines for Surgical Treatment, proposed by the Japanese Association for Operative Medicine,[75] recommend that "the skin be exposed to an antiseptic agent for 2 to 3 minutes to ensure its efficacy." Drying the skin after the application of olanexidine gluconate 1.5% solution before making skin incision is clinically important because this step ensures a sufficient duration of exposure of bacteria on the skin to the solution, and avoids direct exposure of visceral organs to the solution. However, drying the skin after the application of an antiseptic agent before making skin incision is a routine step employed with currently available antiseptic agents, and instructions regarding drying are not included in the wording of the Dosage and Administration of such agents. Further, different lengths of time are required for drying the skin after the application of antiseptics, depending on the skin condition (e.g., hydration status). Thus the Dosage and Administration section for olanexidine gluconate 1.5% solution need not specify how long the skin should be left to dry after application.

In the phase III study (Study 131-301), the mean length of time between the completion of application of the study drug and the start of surgery was 12.2 ± 5.1 minutes (mean ± standard deviation), and the shortest length of time in the olanexidine gluconate 1.5% group was 6 minutes. In order to describe dosage regimen more clearly, the proposed wording for dosage and administration ("Olanedine is applied to the skin") will be changed to "An appropriate amount of Olanedine is applied to the skin."

PMDA’s view:

Neither “the time required for drying the skin” nor “the time required between the application of an antiseptic solution and surgery” is specified in the Dosage and Administration section for any antiseptic agents currently available. Further, different lengths of time are required for drying the skin after the application of antiseptics depending on the skin condition (dehydration status). Therefore, it is difficult to specify the length of time for drying the skin before starting the surgery. In the phase III study (Study 131-301), the length of time between the completion of study drug application and the start of surgery was approximately 12 minutes. This length of time does not differ substantially by type of surgery unless an excessively large skin area must be disinfected. It is thus acceptable not to specify a length of time for drying the skin before the surgery or a length of time between solution application and surgery. However, as it is clinically important to expose the normal skin flora to olanexidine gluconate 1.5% solution for a sufficient length of time and avoid direct exposure of visceral organs to the solution, healthcare professionals should be instructed to dry the skin after the application of olanexidine gluconate solution before starting the surgery.

The above conclusion of PMDA will be discussed at the Expert Discussion.

4.(iii).B.(5) Post-marketing investigations

The applicant plans to conduct the post-marketing surveillance of olanexidine gluconate 1.5% solution (see below for the details). In order to assess the safety of olanexidine gluconate 1.5% solution carefully, the applicant will limit the use of the solution to patients undergoing surgery in selected medical institutions, until safety data are obtained from a certain number of patients (target, 2000 patients). The applicant will promote the proper use of the solution in the selected medical institutions. These medical institutions were selected because they can be frequently visited by applicant's medical representatives who provide relevant information and because they are registered as institutions with Infection Control Premium Categories 1 and 2 in the National Insurance System in Japan. After assessing the initial safety data, the applicant will allow patients in other medical institutions to use the solution to collect safety data (target, additional 3000 patients).

Drug use-results survey

- Objective: To confirm the safety of olanexidine gluconate 1.5% solution in clinical practice in the post-marketing settings

- Population: Patients who received an application of olanexidine gluconate 1.5% solution for the preoperative skin preparation at the surgical site.

- Sample size: 5000 patients (The surveillance will be conducted at selected medical institutions to obtain data for the first 2000 patients receiving various type of surgeries, in order to assess how properly olanexidine gluconate 1.5% solution is used and evaluate its use-results and safety. The surveillance will be then expanded to other medical institutions to collect and assess safety data on olanexidine gluconate 1.5% solution from additional 3000 patients undergoing different surgeries under different conditions.)

Rationale for the sample size:
The target sample size is 5000 patients. The first 2000 patients in selected medical institutions will include 150 patients undergoing cardiovascular surgery, 150 patients undergoing orthopedic surgery, and 300 patients each for non-laparoscopic gastrointestinal surgery, laparoscopic gastrointestinal surgery, and other surgeries (general surgery, neurosurgery, gynecological surgery, urological surgery, and ENT surgery), according to the classification of surgery used in the Surgical Site Infection Surveillance by the Surgical Site Infection Division of the Japan Nosocomial Infections Surveillance.

- Observation period: Patients will be monitored from the time of application of olanexidine gluconate 1.5% solution to 7 days after surgery (for safety evaluation) and 30 days after surgery (for the assessment of SSI)

- Survey period: 6 years

PMDA's view:
The applicant should collect a wide range of information in order to assess how well the instructions and precautions described in the package insert and other documents are followed by healthcare professionals and evaluate how effective these instructions and precautions are in ensuring patient safety. The applicant should also promote the proper use of olanexidine gluconate 1.5% solution and investigate safety profile. These activities by the applicant are required for the following reasons: (1) In the clinical studies, olanexidine gluconate 1.5% solution was evaluated with an extremely limited variety of surgical sites and surgeries. In the post-marketing settings, however, the solution will be applied to various surgical sites in patients undergoing various surgeries. (2) Only limited data are available on the safety of the solution used in various types of surgeries. (3) The solution is not allowed to be applied to damaged skin. (4) Healthcare professionals should be fully instructed not to use the solution to damaged skin or mucosa because other marketed antiseptics are allowed to be applied to damaged skin or mucosa. (5) Other marketed antiseptics are used in hundreds of thousands to millions of patients annually. The solution is thus expected to be used in many patients as well.

Only limited data are available on the use of olanexidine gluconate 1.5% solution in elderly patients, and no data are available on its use in children or pregnant or lactating women. The applicant should collect safety data from such patient populations.

The above conclusion of PMDA will be discussed at 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

Document-based compliance 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. PMDA concluded that there should be no major problems with conducting a regulatory review based on the submitted application documents.

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

A GCP on-site inspection was conducted in accordance with the provisions of the Pharmaceutical Affairs Act for the data submitted in the new drug application (5.3.5.1-2, 5.3.5.1-3, 5.3.5.1-4). PMDA concluded that there should be no major problems with conducting a regulatory review based on the submitted application documents.

IV. Overall Evaluation

The submitted data indicate that olanexidine gluconate 1.5% solution is expected to be effective in the preoperative preparation of intact skin at the surgical site, and its safety is acceptable.

PMDA has concluded that the application may be approved when the Expert Discussion reveals no problems.

Review Report (2)

April 24, 2015

I. Product submitted for registration

[Brand name]	(1) Olanedine Antiseptic Solution 1.5% (2) Olanedine Solution 1.5% Antiseptic Applicator 10 mL; Olanedine Solution 1.5% Antiseptic Applicator 25 mL
[Non-proprietary name]	Olanexidine Gluconate
[Applicant]	Otsuka Pharmaceutical Factory, Inc.
[Date of application]	May 20, 2014

II. Content of the review

The comments from the Expert Discussion and the subsequent review by the Pharmaceuticals and Medical Devices Agency (PMDA) are outlined in the following sections. The expert advisors for the Expert Discussions 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).

In response to the comments from the Expert Discussion, PMDA conducted additional review regarding the following points and took necessary measures. The expert advisors supported all PMDA’s opinions presented in the Review Report (1), except for the following points.

(1) Precautions for application to damaged skin

PMDA’s conclusion regarding the safety of olanexidine gluconate 1.5% solution (see "4.(iii).B.(2) Safety" of the Review Report (1)) was supported by the expert advisors. The following comments were presented by the expert advisors.

- When instructing healthcare professionals not to apply olanexidine gluconate 1.5% solution to surgical wounds or damaged skin, the expression of "wound sites" would be better understood than "damaged skin," because the former is more familiar to healthcare professionals as it is used in the package inserts for currently available antiseptic agents. The type of wound sites should be described with examples to provide more accurate information.
- Healthcare professionals should be instructed to dry the skin after application of olanexidine gluconate 1.5% solution before surgery in order to ensure that normal skin flora are exposed to the solution for sufficient time, and to avoid direct exposure of visceral organs to the solution.

In response to the above comments, PMDA requested the applicant to include the following statements in the

package insert: "The solution should not be applied to wound sites (such as surgical wounds and incisions, and skin with erosion or ulcer)"; and "After applying the solution to the skin, wait until the solution has dried to ensure that the skin is exposed to the solution for sufficient time." The applicant agreed.

(2) Indications

PMDA's conclusion regarding the indication of olanexidine gluconate 1.5% solution (see "4.(iii).B.(3) Indications and clinical positioning" of the Review Report (1)) was supported by the expert advisors. The following comments were presented by the expert advisors.

- Olanexidine gluconate 1.5% solution can be used to eliminate major causative bacteria of procedural site infections as well as normal skin flora on the skin before invasive procedures such as catheterization and punctuation, in the same manner as skin preparation at the surgical sites. As surgeries and invasive procedures may not be clearly distinguished in the clinical setting, the indication of olanexidine gluconate 1.5% solution should not be limited to patients undergoing surgery.
- It is difficult to clearly distinguish surgeries from other invasive procedures. However, healthcare professionals should be informed that olanexidine gluconate 1.5% solution is indicated only for patients undergoing surgery and should not be used in patients undergoing non-surgical invasive procedures, in order to ensure the proper use of the solution, which may cause hypersensitivity and shock.

PMDA discussed this issue in response to the above comments (see below for a summary):
In the post-marketing settings, if olanexidine gluconate 1.5% solution is used to disinfect intact skin for a variety of patients including those undergoing invasive procedures other than surgery, it may be difficult to ensure that healthcare professionals avoid applying the solution to wound sites or mucous membranes and follow other instructions for the proper use. Thus, cautioning healthcare professionals against using the solution in patients undergoing invasive procedures other than surgery seemed one approach to promoting appropriate use. However, considering the current clinical practice in Japan, where surgery and other invasive procedures are not clearly differentiated, it is not practical to provide different instructions for patients undergoing surgery and those undergoing other invasive procedures.

On the basis of the above consideration, PMDA concluded that healthcare professionals need not be cautioned against using the solution in patients undergoing invasive procedures other than surgery, and that the solution should be used at the discretion of healthcare professionals. However, for patient safety, healthcare professionals should be instructed to avoid applying the solution to wound sites or mucous membranes, and to dry the skin after application to ensure that the skin is exposed to the solution for sufficient time. PMDA requested the applicant to provide healthcare professionals with these instructions to ensure the proper use of the solution. The applicant agreed.

(3) The proposed risk management plan

PMDA's conclusion on the post-marketing surveillance (see "4.(iii).B.(5) Post-marketing investigations") was supported by the expert advisors. The expert advisors commented that data on adverse events such as hypersensitivity and shock, which may occur following the application of the solution, should be analyzed for causative factors.

On the basis of the above comment, PMDA considers that the applicant should also collect the following data through the post-marketing surveillance and provide obtained data to healthcare professionals appropriately.

- Safety in elderly patients, children, and pregnant and lactating women
- Relationship between safety and patient characteristics or type of surgical procedures

The applicant should collect data in the post-marketing settings to assess how well healthcare professionals follow the instructions and precautions in the package insert and other documents under various usage conditions, and how effective these instructions and precautions are in ensuring patient safety. The applicant should also continue to collect data on the development of resistance to olanexidine gluconate 1.5% solution, and appropriately provide healthcare professionals with new findings available.

PMDA requested the applicant to consider the above points. The applicant agreed.

In view of the above discussions on the proposed risk management plan, PMDA concluded that the applicant should include the following safety and efficacy specifications in the plan (Table 27) and conduct additional pharmacovigilance activities and additional risk minimization activities (Table 28). The proposed plan for drug use-results survey is presented below (see Table 29).

Table 27. Specifications to be included in the proposed risk management plan in relation to safety and efficacy

Safety specifications		
Important identified risks	Important potential risks	Important missing information
None	Shock	None
Efficacy specifications		
None		

Table 28. Outline of additional pharmacovigilance activities and risk minimization activities in the proposed risk management plan

Additional pharmacovigilance activities	Additional risk minimization activities
- Early post-marketing phase vigilance - Drug use-results survey	- Early post-marketing phase vigilance - Preparing and providing information materials for healthcare professionals (guidance for proper use of olanexidine gluconate)

Table 29. Summary of the proposed drug use-results survey plan

Objective	To confirm the safety of olanexidine gluconate 1.5% solution in the clinical setting in the post-marketing settings, and collect data on surgical site infection.
Survey method	Central registration
Population	Patients who received an application of olanexidine gluconate 1.5% solution for the preoperative skin preparation at the surgical site
Survey period (observation period)	6 years (Patients will be monitored from the time of application of olanexidine gluconate 1.5% solution to 7 days after surgery [for safety evaluation] and 30 days after surgery [for the assessment of SSI].)
Planned sample size	5000 patients
Major survey items	Patient characteristics, usage of olanexidine gluconate 1.5% solution (e.g., amount applied, purpose, application site), adverse events, concomitant medication, details of the surgery (e.g., duration, pre-operative status, type of wound, type of surgical procedure, and incision length), the presence/absence of surgical site infection

III. Overall Evaluation

As a result of the above review, PMDA has concluded that the product may be approved with the following approval conditions and the indication after modifying the dosage and administration as shown below. The re-examination period is 8 years because the product is a drug with a new active ingredient. Neither the drug substance nor the drug product is classified as a poisonous drug, powerful drug, biological product, or specified biological product.

[Indications] Preoperative skin preparation at the surgical site

[Dosage and administration] An appropriate amount of Olanedine is applied to the skin.

[Conditions for approval] The applicant is required to develop and appropriately implement a risk management plan.