2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: M4, M9, M10

Reversible Inhibition of CYPs In Vitro (Lemborexant metabolites) **Study Number: B130772** **Location in CTD: 5.3.2.2.9**

Study System	in vitro			
Test System	Human liver microsomes (0.2 mg protein/mL) in the presence of an NADPH-generating system			
Method	Incubation of each metabolite with each probe substrate in microsomal matrix at 37 °C for designated time			
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP mediated metabolism			
Concentration	3, 10, and 30 µmol/L			
Percentage Inhibition on CYP Isoform-Specific Activity by M4 at 30 µmol/L and Positive Control Inhibitors				
CYP Isoform (Substrate)	**Inhibition (%)[a]**			
	M4	**Positive Control Inhibition**		
	30 µmol/L	**Inhibitor**	**Conc. (µmol/L)**	**Inhibition (%)[a]**
CYP1A2 (Phenacetin)	–3.2	α-Naphthoflavone	0.02	52.9
CYP2A6 (Coumarin)	7.3	8-Methoxypsoralen	1	54.3
CYP2B6 (Bupropion)	11.8	Ticlopidine	0.2	44.1
CYP2C8 (Paclitaxel)	12.7	Montelukast	0.2	53.8
CYP2C9 (Diclofenac)	38.4	Sulfaphenazole	0.5	50.0
CYP2C19 ((*S*)-Mephenytoin)	38.8	Tranylcypromine	10	63.7
CYP2D6 (Bufurarol)	12.6	Quinidine	0.1	62.6
CYP2E1 (Chlorzoxazone)	–93.5	Diethyldithiocarbamic acid	4	35.9
CYP3A (Midazolam)	27.1	Ketoconazole	0.02	40.7
CYP3A (Testosterone)	19.4	Ketoconazole	0.02	35.2
CYP3A (Nifedipine)	17.2	Ketoconazole	0.02	39.7

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Test Article: M4, M9, M10

Reversible Inhibition of CYPs In Vitro (Lemborexant metabolites) **Study Number: B130772** **Location in CTD: 5.3.2.2.9**

Percentage Inhibition on CYP Isoform-Specific Activity by M9 at 30 µmol/L and Positive Control Inhibitors				
CYP Isoform (Substrate)	**Inhibition (%)[a]**			
	M9	**Positive Control Inhibition**		
	30 µmol/L	**Inhibitor**	**Conc. (µmol/L)**	**Inhibition (%)[a]**
CYP1A2 (Phenacetin)	2.9	α-Naphthoflavone	0.02	52.9
CYP2A6 (Coumarin)	21.3	8-Methoxypsoralen	1	54.3
CYP2B6 (Bupropion)	15.4	Ticlopidine	0.2	44.1
CYP2C8 (Paclitaxel)	18.2	Montelukast	0.2	53.8
CYP2C9 (Diclofenac)	37.9	Sulfaphenazole	0.5	50.0
CYP2C19 ((*S*)-Mephenytoin)	39.9	Tranylcypromine	10	63.7
CYP2D6 (Bufurarol)	11.7	Quinidine	0.1	62.6
CYP2E1 (Chlorzoxazone)	–51.0	Diethyldithiocarbamic acid	4	35.9
CYP3A (Midazolam)	11.5	Ketoconazole	0.02	40.7
CYP3A (Testosterone)	18.3	Ketoconazole	0.02	35.2
CYP3A (Nifedipine)	0.6	Ketoconazole	0.02	39.7

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Test Article: M4, M9, M10

Reversible Inhibition of CYPs In Vitro (Lemborexant metabolites) **Study Number: B130772** **Location in CTD: 5.3.2.2.9**

Percentage Inhibition on CYP Isoform-Specific Activity by M10 at 30 µmol/L and Positive Control Inhibitors

CYP Isoform (Substrate)	Inhibition (%)[a]			
	M10	Positive Control Inhibition		
	30 µmol/L	Inhibitor	Conc. (µmol/L)	Inhibition (%)[a]
CYP1A2 (Phenacetin)	0.9	α-Naphthoflavone	0.02	52.9
CYP2A6 (Coumarin)	35.8	8-Methoxypsoralen	1	54.3
CYP2B6 (Bupropion)	11.8	Ticlopidine	0.2	44.1
CYP2C8 (Paclitaxel)	12.7	Montelukast	0.2	53.8
CYP2C9 (Diclofenac)	36.8	Sulfaphenazole	0.5	50.0
CYP2C19 ((*S*)-Mephenytoin)	36.4	Tranylcypromine	10	63.7
CYP2D6 (Bufurarol)	18.7	Quinidine	0.1	62.6
CYP2E1 (Chlorzoxazone)	–57.7	Diethyldithiocarbamic acid	4	35.9
CYP3A (Midazolam)	35.3	Ketoconazole	0.02	40.7
CYP3A (Testosterone)	27.6	Ketoconazole	0.02	35.2
CYP3A (Nifedipine)	26.8	Ketoconazole	0.02	39.7

Conc. = concentration, CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

a: The remaining activity and inhibition (%) of each CYP isoform were calculated in the reversible inhibition assay.

Remaining activity (%) = As / Ac × 100.

Where As = metabolic activity of test substance samples and positive control samples and Ac = metabolic activity of the respective vehicle control.

Inhibition (%) = 100 – Remaining activity.

Additional Information: M4, M9, and M10 showed a weak activating effect on CYP2E1. Inhibition (%) at 3, 10, and 30 µmol/L were –18.6%, –59.6%, and –93.5% for M4, –7.8%, –25.9%, and –51.0% for M9, and –17.9%, –44.5%, and –57.7% for M10, respectively.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: M4, M9, M10

Time Dependent Inhibition of CYPs In Vitro (Lemborexant metabolites) **Study Number: B130772** **Location in CTD: 5.3.2.2.9**

Study System	in vitro
Test System	Human liver microsomes in the presence of a NADPH-generating system
Method	Lemborexant was pre-incubated with human liver microsomes at 37 °C for 30 minutes prior to addition of the appropriate CYP substrate.
Assay / Analyte	LC-MS/MS / Metabolites formed by CYP mediated metabolism
Concentration	3, 10, and 30 µmol/L

Time-dependent Inhibition on CYP Isoform-specific Activity by M4 at 30 µmol/L and Positive Control Inhibitors

CYP Isoform (Substrate)	**M4 (30 µmol/L)**	**Positive Control**		
	% Activity Loss[a]	**Inhibitor**	**Conc. (µmol/L)**	**% Activity Loss[a]**
CYP1A2 (Phenacetin)	3.4	Furafylline	10	67.5
CYP2A6 (Coumarin)	–3.7	8-Methoxypsoralene	1	79.6
CYP2B6 (Bupropion)	4.5	Ticlopidine	2	65.3
CYP2C8 (Paclitaxel)	0.3	Gemfibrozil 1-*O*-β-glucuronide	30	63.5
CYP2C9 (Diclofenac)	–1.1	Tienilic acid	10	60.8
CYP2C19 ((*S*)-mephenytoin)	–1.9	*S*-Fluoxetine	100	57.1
CYP2D6 (Bufurarol)	2.6	Paroxetine	10	52.3
CYP2E1 (Chlorzoxazone)	8.1	Diethyldithiocarbamic acid	10	51.0
CYP3A (Midazolam)	26.6	Verapamil	20	75.1

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Test Article: M4, M9, M10

Time Dependent Inhibition of CYPs In Vitro (Lemborexant metabolites)　**Study Number: B130772**　**Location in CTD: 5.3.2.2.9**

Time-dependent Inhibition on CYP Isoform-specific Activity by M9 at 30 µmol/L and Positive Control Inhibitors				
CYP Isoform (Substrate)	**M9 (30 µmol/L)**	**Positive Control**		
	% Activity Loss[a]	**Inhibitor**	**Conc. (µmol/L)**	**% Activity Loss[a]**
CYP1A2 (Phenacetin)	0.7	Furafylline	10	67.5
CYP2A6 (Coumarin)	–4.1	8-Methoxypsoralene	1	79.6
CYP2B6 (Bupropion)	7.0	Ticlopidine	2	65.3
CYP2C8 (Paclitaxel)	–0.3	Gemfibrozil 1-*O*-β-glucuronide	30	63.5
CYP2C9 (Diclofenac)	0.7	Tienilic acid	10	60.8
CYP2C19 ((*S*)-mephenytoin)	0.1	*S*-Fluoxetine	100	57.1
CYP2D6 (Bufurarol)	2.7	Paroxetine	10	52.3
CYP2E1 (Chlorzoxazone)	3.2	Diethyldithiocarbamic acid	10	51.0
CYP3A (Midazolam)	18.8	Verapamil	20	75.1

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Test Article: M4, M9, M10

Time Dependent Inhibition of CYPs In Vitro (Lemborexant metabolites)　**Study Number: B130772**　**Location in CTD: 5.3.2.2.9**

Time-dependent Inhibition on CYP Isoform-specific Activity by M10 at 30 µmol/L and Positive Control Inhibitors				
CYP Isoform (Substrate)	**M10 (30 µmol/L)**	**Positive Control**		
	% Activity Loss[a]	**Inhibitor**	**Conc. (µmol/L)**	**% Activity Loss[a]**
CYP1A2 (Phenacetin)	11.1	Furafylline	10	67.5
CYP2A6 (Coumarin)	–31.6	8-Methoxypsoralene	1	79.6
CYP2B6 (Bupropion)	3.1	Ticlopidine	2	65.3
CYP2C8 (Paclitaxel)	–10.0	Gemfibrozil 1-*O*-β-glucuronide	30	63.5
CYP2C9 (Diclofenac)	–2.9	Tienilic acid	10	60.8
CYP2C19 ((*S*)-mephenytoin)	1.1	*S*-Fluoxetine	100	57.1
CYP2D6 (Bufurarol)	0.1	Paroxetine	10	52.3
CYP2E1 (Chlorzoxazone)	9.1	Diethyldithiocarbamic acid	10	51.0
CYP3A (Midazolam)	34.7	Verapamil	20	75.1

Details of the time-dependent inhibition measurement method: Each metabolite was pre-incubated for 30 minutes with human liver microsomes (2 mg protein/mL) in the absence and presence of NADPH-generating system, and then the mixtures were diluted 10-fold and incubated with each substrate in the presence of NADPH-generating system.

Conc. = concentration, CTD = Common Technical Document, CYP = cytochrome P450, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NADPH = reduced form of β-nicotinamide adenine dinucleotide phosphate.

a: The % activity loss was calculated from the following equation in time-dependent inhibition assay

The % activity loss = $100 \times ([As / Ac]_{\text{without NADPH}} - [As / Ac]_{\text{with NADPH}})$

Where As = metabolic activity of test substance samples and positive control samples and Ac = metabolic activity of the respective vehicle control.

2.6.5.12 薬物動態試験：薬物代謝酵素の誘導/阻害（続き）

Test Article: lemborexant, M4, M9, M10

Induction of CYPs In Vitro **Study Number: 15-131** **Location in CTD: 5.3.2.2.10**

Study System	in vitro		
Test System	Primary culture system of human hepatocytes from 3 donors (Lot Nos. IPH, ONO, and QOQ)		
Method	Cell cultures, treatment once daily for 3 consecutive days at 37 °C		
Assay/ Analyte	qRT-PCR/ mRNA		
Concentration	0.3, 1, 3, 10, 30, and 80 µmol/L as lemborexant, and 1, 3, and 10 µmol/L as M4, M9, or M10		
Test Article or Positive Control Inducer (concentration)	**Fold Induction on CYP1A2 mRNA After Treatment With Omeprazole and Lemborexant**		
	Lot No. IPH	**Lot No. ONO**	**Lot No. QOQ**
Omeprazole (50 µmol/L)	31.68	31.09	20.52
Lemborexant (0.3 µmol/L)	1.78	1.41	1.64
Lemborexant (1 µmol/L)	1.77	1.69	1.60
Lemborexant (3 µmol/L)	2.06	1.73	1.74
Lemborexant (10 µmol/L)	2.46	2.08	2.11
Lemborexant (30 µmol/L)	3.65	2.68	3.36
Lemborexant (80 µmol/L)	5.28	3.29	3.28

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Test Article: lemborexant, M4, M9, M10

Induction of CYPs In Vitro　　**Study Number: 15-131**　　**Location in CTD: 5.3.2.2.10**

Test Article or Positive Control Inducer (concentration)	**Fold Induction on CYP2B6 mRNA After Treatment With Phenobarbital and Lemborexant**		
	Lot No. IPH	**Lot No. ONO**	**Lot No. QOQ**
Phenobarbital (1000 µmol/L)	7.51	8.49	7.39
Lemborexant (0.3 µmol/L)	2.20	1.95	2.25
Lemborexant (1 µmol/L)	1.89	1.80	1.87
Lemborexant (3 µmol/L)	2.24	2.34	2.41
Lemborexant (10 µmol/L)	3.83	3.07	3.12
Lemborexant (30 µmol/L)	5.87	4.64	5.02
Lemborexant (80 µmol/L)	6.49	4.74	4.02
Test Article or Positive Control Inducer (concentration)	**Fold Induction on CYP3A4 mRNA After Treatment With Rifampicin and Lemborexant**		
	Lot No. IPH	**Lot No. ONO**	**Lot No. QOQ**
Rifampicin (20 µmol/L)	30.25	115.83	45.40
Lemborexant (0.3 µmol/L)	1.70	1.84	1.91
Lemborexant (1 µmol/L)	2.42	3.69	2.49
Lemborexant (3 µmol/L)	5.40	9.95	4.35
Lemborexant (10 µmol/L)	13.73	23.32	8.52
Lemborexant (30 µmol/L)	20.61	43.30	14.15
Lemborexant (80 µmol/L)	24.20	38.96	13.44

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Test Article: lemborexant, M4, M9, M10

Induction of CYPs In Vitro　　**Study Number: 15-131**　　**Location in CTD: 5.3.2.2.10**

CYP Isoform	Lemborexant Concentration (μmol/L)	% of Positive Control on CYP1A2, CYP2B6, and CYP3A4 mRNA After Treatment With Lemborexant		
		Lot No. IPH	Lot No. ONO	Lot No. QOQ
CYP1A2	0.3	2.5	1.4	3.3
	1	2.5	2.3	3.1
	3	3.5	2.4	3.8
	10	4.8	3.6	5.7
	30	8.6	5.6	12.1
	80	13.9	7.6	11.7
CYP2B6	0.3	18.5	12.7	19.6
	1	13.7	10.7	13.7
	3	19.1	17.9	22.0
	10	43.5	27.6	33.2
	30	74.8	48.5	62.9
	80	84.4	49.9	47.3
CYP3A4	0.3	2.4	0.7	2.1
	1	4.9	2.3	3.3
	3	15.0	7.8	7.5
	10	43.5	19.4	16.9
	30	67.0	36.8	29.6
	80	79.3	33.1	28.0

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Test Article: lemborexant, M4, M9, M10

Induction of CYPs In Vitro **Study Number: 15-131** **Location in CTD: 5.3.2.2.10**

Induction Parameters of Lemborexant on CYP3A4		
Lot No.	**E_{max} (fold)**	**EC_{50} (µmol/L)**
IPH	25.86	9.91
ONO	43.10	7.88
QOQ	14.90	7.44

Test Article or Positive Control Inducer (concentration)	**Fold Induction on CYP1A2 mRNA After Treatment With Omeprazole and Lemborexant Metabolites**			**% of Positive Control on CYP1A2 mRNA After Treatment With Lemborexant Metabolites**		
	Lot No. IPH	**Lot No. ONO**	**Lot No. QOQ**	**Lot No. IPH**	**Lot No. ONO**	**Lot No. QOQ**
Omeprazole (50 µmol/L)	38.79	19.37	22.93	NA	NA	NA
M4 (1 µmol/L)	1.82	1.60	1.53	2.2	3.2	2.4
M4 (3 µmol/L)	1.55	1.67	1.70	1.5	3.6	3.2
M4 (10 µmol/L)	1.93	1.73	1.85	2.5	4.0	3.9
M9 (1 µmol/L)	1.52	1.49	1.41	1.4	2.7	1.9
M9 (3 µmol/L)	1.58	1.63	1.37	1.5	3.4	1.7
M9 (10 µmol/L)	1.17	1.64	1.34	0.5	3.5	1.5
M10 (1 µmol/L)	1.78	1.74	1.54	2.1	4.0	2.5
M10 (3 µmol/L)	2.03	1.89	1.79	2.7	4.8	3.6
M10 (10 µmol/L)	2.17	2.42	2.25	3.1	7.7	5.7

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Test Article: lemborexant, M4, M9, M10

Induction of CYPs In Vitro　　**Study Number: 15-131**　　**Location in CTD: 5.3.2.2.10**

Test Article or Positive Control Inducer (concentration)	Fold Induction on CYP2B6 mRNA After Treatment With Phenobarbital and Lemborexant Metabolites			% of Positive Control on CYP2B6 mRNA After Treatment With Lemborexant Metabolites		
	Lot No. IPH	Lot No. ONO	Lot No. QOQ	Lot No. IPH	Lot No. ONO	Lot No. QOQ
Phenobarbital (1000 µmol/L)	15.65	7.79	8.79	NA	NA	NA
M4 (1 µmol/L)	1.33	1.87	1.86	2.3	12.8	11.0
M4 (3 µmol/L)	2.09	2.39	2.15	7.5	20.5	14.7
M4 (10 µmol/L)	2.94	2.92	2.65	13.2	28.3	21.2
M9 (1 µmol/L)	1.46	1.40	1.50	3.1	5.9	6.5
M9 (3 µmol/L)	1.76	1.70	1.25	5.2	10.3	3.2
M9 (10 µmol/L)	2.44	2.39	2.05	9.8	20.5	13.5
M10 (1 µmol/L)	1.28	1.96	1.65	1.9	14.1	8.4
M10 (3 µmol/L)	2.06	2.23	2.32	7.3	18.2	16.9
M10 (10 µmol/L)	2.56	3.84	3.65	10.6	41.8	34.1

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Test Article: lemborexant, M4, M9, M10

Induction of CYPs In Vitro **Study Number: 15-131** **Location in CTD: 5.3.2.2.10**

Test Article or Positive Control Inducer (concentration)	Fold Induction on CYP3A4 mRNA After Treatment With Rifampicin and Lemborexant Metabolites			% of Positive Control on CYP3A4 mRNA After Treatment With Lemborexant Metabolites		
	Lot No. IPH	Lot No. ONO	Lot No. QOQ	Lot No. IPH	Lot No. ONO	Lot No. QOQ
Rifampicin (20 µmol/L)	37.37	131.16	46.08	NA	NA	NA
M4 (1 µmol/L)	2.89	2.05	1.81	5.2	0.8	1.8
M4 (3 µmol/L)	3.98	4.14	2.75	8.2	2.4	3.9
M4 (10 µmol/L)	12.17	12.75	4.15	30.7	9.0	7.0
M9 (1 µmol/L)	2.02	2.02	2.06	2.8	0.8	2.3
M9 (3 µmol/L)	3.23	3.76	2.50	6.1	2.1	3.3
M9 (10 µmol/L)	9.12	14.13	5.49	22.3	10.1	10.0
M10 (1 µmol/L)	2.62	2.21	1.67	4.5	0.9	1.5
M10 (3 µmol/L)	7.26	4.44	3.39	17.2	2.6	5.3
M10 (10 µmol/L)	19.47	15.65	12.69	50.8	11.3	25.9

Fold induction and response as a percentage of positive control in each lot represents the mean value (n=3). Omeprazole, phenobarbital, and rifampicin were used as positive control inducers for CYP1A2, CYP2B6, and CYP3A4, respectively.

CTD = Common Technical Document, CYP = cytochrome P450, EC_{50} = concentration causing half the maximal effect, E_{max} = maximum induction effect, mRNA = messenger RNA, NA = not applicable, qRT-PCR = quantitative reverse transcriptase polymerase chain reaction.

Additional Information: Lemborexant (0.3 to 80 µmol/L) and its metabolites (M4, M9, and M10; 1 to 10 µmol/L) showed potent concentration-dependent inductive effects on mRNA levels of CYP2B6 and CYP3A4. Lemborexant also slightly induced CYP1A2 mRNA concentration-dependently at 3 µmol/L or higher (up to 80 µmol/L). M4, M9, and M10 showed no or minimal inductive effects on CYP1A2 mRNA. In all experiments, no significant cytotoxic effects by test articles and positive control inducers were observed.

2.6.5.13 薬物動態試験：排泄

Test Article: [^{14}C]lemborexant

Rats **Study Number: 14-074** **Location in CTD: 4.2.2.3.1**

Strain/Gender/Number of Animals	Sprague Dawley/Male/3		
Feeding Condition	Fasted[a]		
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution		
Method of Administration / Dose	Oral, by using a polypropylene syringe with a gavage needle / 10 mg/kg		
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (K0110-030)		
Analyte/Assay	Radioactivity/Liquid scintillation counting		
Time (h)	**Cumulative Excretion of Radioactivity (% of dose)**		
	Urine	**Feces**	**Total**
0 – 24	5.6±0.7	88.8±1.8	94.4±1.2
0 – 48	5.8±0.7	90.2±1.2	96.1±0.6
0 – 72	5.9±0.8	90.4±1.2	96.2±0.6
0 – 96	5.9±0.8	90.5±1.2	96.3±0.6
0 – 120	5.9±0.8	90.6±1.2	96.4±0.6
0 – 144	5.9±0.8	90.6±1.2	96.5±0.6
0 – 168	5.9±0.8	90.6±1.2	96.5±0.6

Values represent the mean ±SEM of 3 animals.

CTD = Common Technical Document.

a: The fasting of the animals was started at 17:30 to 18:30 on the day before dosing (at least 16 hours before dosing) and terminated at 6 hours after administration. Water was given freely.

Additional Information: This study also investigated the blood level of radioactivity (Section 2.6.5.3), the distribution of radioactivity in organs and tissues (Section 2.6.5.5), and the biliary excretion of radioactivity (Section 2.6.5.14).

2.6.5.13 薬物動態試験：排泄（続き）

Test Article: [^{14}C]lemborexant

Monkeys **Study Number: 14-075** **Location in CTD: 4.2.2.3.2**

Strain/Gender/Number of Animals	Cynomolgus/Male/3		
Feeding Condition	Fasted[a]		
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution		
Method of Administration / Dose	Oral, by using a polypropylene syringe with catheter / 3 mg/kg		
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (K0110-030)		
Analyte/Assay	Radioactivity/Liquid scintillation counting		
Time (h)	**Cumulative Excretion of Radioactivity (% of dose)**		
	Urine	**Feces**	**Total**
0 – 24	15.1±0.5	4.5±4.2	19.6±3.7
0 – 48	18.5±0.6	43.0±3.1	61.4±3.1
0 – 72	20.2±1.0	62.2±4.0	82.3±3.0
0 – 96	21.1±1.1	67.8±3.6	88.9±2.5
0 – 120	21.7±1.3	71.1±2.4	92.8±1.1
0 – 144	22.0±1.4	72.8±1.6	94.8±0.3
0 – 168	22.3±1.5	73.8±1.2	96.1±0.4
0 – 192	22.4±1.6	74.4±1.0	96.8±0.5
0 – 216	22.4±1.6	74.7±0.9	97.1±0.7
0 – 240	22.4±1.6	74.9±0.9	97.3±0.7

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Test Article: **[^{14}C]lemborexant**

Monkeys **Study Number: 14-075** **Location in CTD: 4.2.2.3.2**

Time (h)	Cumulative Excretion of Radioactivity (% of dose)		
	Urine	Feces	Total
0 – 264	22.5±1.6	75.0±0.8	97.4±0.8
0 – 288	22.5±1.6	75.0±0.8	97.5±0.8
0 – 316	22.5±1.6	75.1±0.8	97.5±0.9
0 – 336	22.5±1.6	75.1±0.8	97.5±0.9

Values represent the mean ±SEM of 3 animals.

CTD = Common Technical Document.

a: The fasting of the animals was started from 18:00 on the day before the dosing (at least 16 hours before dosing) to 6 hours after the dosing. Water was given freely.

Additional Information: This study also investigated the blood level of radioactivity (Section 2.6.5.3) and the distribution of radioactivity in organs and tissues (Section 2.6.5.5).

2.6.5.14 薬物動態試験：排泄：胆汁中

Test Article: [^{14}C]lemborexant

Rats (Bile duct-cannulated) **Study Number: 14-074** **Location in CTD: 4.2.2.3.1**

Strain/Gender/Number of Animals	Sprague Dawley/Male/3			
Feeding Condition	Fasted[a]			
Vehicle / Formulation	100 mmol/L hydrochloric acid / Solution			
Method of Administration / Dose	Oral, by using a polypropylene syringe with a gavage needle / 10 mg/kg			
Radionuclide / Specific Activity (Lot No.)	[^{14}C] / [redacted] MBq/mg (K0110-030)			
Analyte/Assay	Radioactivity/Liquid scintillation counting			
Time (h)	**Cumulative Excretion of Radioactivity (% of dose)**			
	Bile	**Urine**	**Feces**	**Total**
0 – 2	80.3±4.8	—	—	—
0 – 4	93.0±2.8	—	—	—
0 – 6	96.7±2.0	—	—	—
0 – 8	98.1±1.4	—	—	—
0 – 12	99.0±1.1	—	—	—
0 – 24	99.8±0.8	1.1±0.2	1.1±0.1	102.0±0.5
0 – 48	100.0±0.7	1.1±0.2	1.2±0.1	102.3±0.3

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Test Article: **[^{14}C]lemborexant**

Rats (Bile duct-cannulated) **Study Number: 14-074** **Location in CTD: 4.2.2.3.1**

Residual	Residual Radioactivity (% of dose)
Gastrointestinal contents (48 h)	0.0±0.0
Carcass (48 h)	0.9±0.2

Values represent the mean ±SEM of 3 animals.

— = Not collected, CTD = Common Technical Document.

a: The fasting of the animals was started at 17:30 to 18:30 on the day before dosing (at least 16 hours before dosing) and terminated at 6 hours after administration. Water was given freely.

Additional Information: This study also investigated the blood level of radioactivity (Section 2.6.5.3), the distribution of radioactivity in organs and tissues (Section 2.6.5.5), and the excretion of radioactivity in urine and feces (Section 2.6.5.13).

2.6.5.15 薬物動態試験：薬物相互作用

非臨床 in vivo における薬物間相互作用試験は検討されていない。

一方，in vitro における薬物間相互作用（CYP 誘導及び CYP 阻害を含む）にかかわる試験はヒト生体試料を用いて評価した（2.6.5.12 及び 2.7.2.1.1 項）。ヒトトランスポーターの基質となるか，又はそれらを阻害する可能性については，2.6.5.16 及び 2.7.2.1.1 項に記した。

2.6.5.16 薬物動態試験：その他

Test Article: lemborexant, M4, M9, M10, M13, M14, M15

Transport by P-gp　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Study System	in vitro
Test System	Cultured monolayers of LLC-PK1 cells and human P-gp expressing LLC-PK1 (LLC-MDR1) cells
Method	Incubation of each test article with cell monolayers for 2 hours at 37 °C
Assay / Analyte / Concentration	LC-MS/MS / lemborexant, M4, M9, M10, M13, M14, or M15 / 3 µmol/L as each test article

Transcellular Transport of lemborexant, M4, M9, and M10 in P-gp Expressing Cells and Its Control Cells

Compound	Concentration (µmol/L)	Verapamil (30 µmol/L)	LLC-MDR1			LLC-PK1			CFR (1)
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	
Lemborexant	3	–	23.25±2.60	30.43±1.39	1.31	31.69±0.31	28.33±0.91	0.89	1.47
		+	31.13±1.41	30.77±1.35	0.99	34.95±2.20	29.70±0.17	0.85	1.16
M4	3	–	2.03±0.35	32.34±2.51	15.93	23.04±1.38	22.67±0.93	0.98	16.26
		+	10.64±0.70	23.99±1.99	2.25	21.88±2.80	21.16±0.64	0.97	2.32
M9	3	–	8.40±0.99	28.36±2.49	3.38	18.77±2.98	22.11±1.39	1.18	2.86
		+	18.69±2.28	24.03±0.89	1.29	21.95±2.20	23.64±1.95	1.08	1.19
M10	3	–	9.25±0.38	36.74±1.37	3.97	29.39±0.55	26.38±0.76	0.90	4.41
		+	26.24±2.61	27.52±1.39	1.05	28.50±1.37	24.47±0.42	0.86	1.22
[^{3}H]Quinidine	1	–	1.00±0.10	26.60±0.87	26.60	11.96±0.69	15.94±0.41	1.33	20.00
		+	12.62±0.29	19.87±0.30	1.57	17.18±0.88	16.06±0.24	0.93	1.69
[^{3}H]Digoxin	1	–	0.64±0.13	9.53±0.16	14.89	2.42±0.12	5.89±0.16	2.43	6.13
		+	2.02±0.11	7.99±0.08	3.96	3.85±0.17	4.22±0.28	1.10	3.60
[^{14}C]Mannitol	1.75	–	1.47±0.26	1.36±0.04	NA	1.57±0.36	1.88±0.28	NA	NA

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Test Article: lemborexant, M4, M9, M10, M13, M14, M15

Transport by P-gp　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Transcellular Transport of M13, M14, and M15 in P-gp Expressing Cells and Its Control Cells

Compound	Concentration (μmol/L)	Verapamil (30 μmol/L)	LLC-MDR1			LLC-PK1			CFR (1)
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	
M13	3	–	0.88±0.14	26.82±1.28	30.48	8.80±1.09	15.02±0.20	1.71	17.82
		+	3.48±0.61	19.18±0.55	5.51	11.95±1.54	11.78±0.28	0.99	5.57
M14	3	–	0.98±0.02	25.56±1.39	26.08	10.19±1.29	16.54±0.79	1.62	16.10
		+	4.81±0.45	22.04±0.73	4.58	12.78±0.40	14.34±0.90	1.12	4.09
M15	3	–	2.66±0.07	30.95±1.20	11.64	22.52±2.65	23.02±0.94	1.02	11.41
		+	12.95±1.10	27.84±2.73	2.15	22.17±2.16	21.27±2.17	0.96	2.24
[^{3}H]Quinidine	1	–	1.01±0.04	28.49±0.84	28.21	11.90±1.05	17.47±0.25	1.47	19.19
		+	12.99±0.40	19.62±0.26	1.51	16.05±0.23	16.42±0.67	1.02	1.48
[^{3}H]Digoxin	1	–	0.62±0.05	8.78±0.22	14.16	1.83±0.06	5.14±0.06	2.81	5.04
		+	1.97±0.05	7.59±0.23	3.85	3.38±0.70	3.88±0.01	1.15	3.35
[^{14}C]Mannitol	1.75	–	1.86±0.35	1.73±0.15	NA	1.85±0.16	1.93±0.24	NA	NA

Each P_{app} value represents the mean ±SD of 3 samples.　LLC-MDR1:　P-gp expressing cell, LLC-PK1:　control cell.

[^{3}H]Quinidine and [^{3}H]digoxin are positive controls for transcellular transport for P-gp.　[^{14}C]Mannitol is a pallacellular flux marker.　Verapamil (30 μmol/L) is a typical P-gp inhibitor.

$P_{app\ A\ to\ B}$ = P_{app} value from the apical to the basal side ($\times 10^{-6}$ cm/sec).

$P_{app\ B\ to\ A}$ = P_{app} value from the basal to the apical side ($\times 10^{-6}$ cm/sec).

FR = $P_{app\ B\ to\ A}$ / $P_{app\ A\ to\ B}$.

CFR (1) = FR in LLC-MDR1 / FR in LLC-PK1.

CFR = corrected flux ratio, CTD = Common Technical Document, FR = flux ratio, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NA = not applicable, P_{app} = apparent permeability, P-gp = P-glycoprotein.

Additional Information:　Lemborexant was a poor substrate for P-gp.　The metabolites M4, M9, M10, M13, M14, and M15 were the substrates of P-gp.

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Test Article: lemborexant, M4, M9, M10, M13, M14, M15

Transport by BCRP　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Study System	in vitro
Test System	Cultured monolayers of MDCK II cells and human BCRP expressing MDCK II (MDCK II-BCRP) cells
Method	Incubation of each test article with cell monolayers for 2 hours at 37 °C
Assay / Analyte / Concentration	LC-MS/MS / lemborexant, M4, M9, M10, M13, M14, or M15 / 3 μmol/L as each test article

Transcellular Transport of lemborexant, M4, M9, and M10 in BCRP Expressing Cells and Its Control Cells

Compound	**Concentration (μmol/L)**	**Ko143 (0.05 μmol/L)**	**MDCK II-BCRP**			**MDCK II**			**CFR (1)**
			$P_{app\ A\ to\ B}$	**$P_{app\ B\ to\ A}$**	**Flux Ratio**	**$P_{app\ A\ to\ B}$**	**$P_{app\ B\ to\ A}$**	**Flux Ratio**	
Lemborexant	3	–	21.29±0.39	27.34±2.60	1.28	28.54±1.93	28.58±0.76	1.00	1.28
		+	22.33±2.51	25.55±1.22	1.14	27.39±3.00	25.83±1.53	0.94	1.21
M4	3	–	6.97±1.38	25.54±0.58	3.66	10.07±0.51	26.02±0.09	2.58	1.42
		+	7.17±0.71	21.92±0.18	3.06	9.60±0.06	24.22±1.99	2.52	1.21
M9	3	–	17.39±0.94	22.93±1.20	1.32	18.43±1.62	23.41±1.67	1.27	1.04
		+	17.69±1.59	24.04±1.36	1.36	18.72±1.78	22.19±1.33	1.19	1.14
M10	3	–	23.06±0.93	24.81±1.80	1.08	21.54±0.81	24.82±0.66	1.15	0.94
		+	20.50±1.16	24.54±0.63	1.20	21.00±1.37	25.47±1.04	1.21	0.99
[^{3}H]Prazosin	1	–	1.51±0.19	18.58±1.09	12.30	8.34±0.50	12.75±1.09	1.53	8.04
		+	5.12±0.41	15.74±0.74	3.07	8.42±1.46	11.95±0.79	1.42	2.16
[^{14}C]Mannitol	1.75	–	1.28±0.06	0.99±0.06	NA	1.32±0.67	1.06±0.10	NA	NA

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Test Article: **lemborexant, M4, M9, M10, M13, M14, M15**

Transport by BCRP **Study Number:** **DMPKT2013-010** **Location in CTD:** **5.3.2.3.2**

Transcellular Transport of M13, M14, and M15 in BCRP Expressing Cells and Its Control Cells

Compound	Concentration (μmol/L)	Ko143 (0.05 μmol/L)	MDCK II-BCRP			MDCK II			CFR (1)
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	
M13	3	–	2.39±0.21	18.15±0.42	7.59	2.51±0.50	18.01±0.59	7.18	1.06
		+	2.48±0.52	16.55±1.85	6.67	3.22±0.44	17.20±0.33	5.34	1.25
M14	3	–	3.73±0.41	19.97±1.22	5.35	3.20±0.26	20.16±0.29	6.30	0.85
		+	4.17±0.91	18.98±0.39	4.55	3.44±0.54	19.98±0.37	5.81	0.78
M15	3	–	9.20±0.54	21.45±1.20	2.33	7.89±0.83	23.30±1.35	2.95	0.79
		+	9.60±1.00	21.26±1.27	2.21	8.66±0.50	22.56±2.39	2.61	0.85
[^{3}H]Prazosin	1	–	1.42±0.14	15.62±0.62	11.00	8.59±0.76	11.16±0.04	1.30	8.46
		+	4.80±0.69	14.75±1.40	3.07	8.15±0.18	11.90±0.88	1.46	2.10
[^{14}C]Mannitol	1.75	–	1.65±0.51	1.82±0.16	NA	1.52±0.13	1.93±0.05	NA	NA

Each P_{app} value represents the mean ±SD of 3 samples. MDCK II-BCRP: BCRP expressing cell, MDCK II: control cell.

[^{3}H]Prazosin is a positive control for transcellular transport for BCRP. [^{14}C]Mannitol is a pallacellular flux marker. Ko143 (0.05 μmol/L) is a typical BCRP inhibitor.

$P_{app\ A\ to\ B}$ = P_{app} value from the apical to the basal side ($\times 10^{-6}$ cm/sec).

$P_{app\ B\ to\ A}$ = P_{app} value from the basal to the apical side ($\times 10^{-6}$ cm/sec).

FR = $P_{app\ B\ to\ A}$ / $P_{app\ A\ to\ B}$.

CFR (1) = FR in MDCK II-BCRP / FR in MDCK II.

BCRP = breast cancer resistant protein, CFR = corrected flux ratio, CTD = Common Technical Document, FR = flux ratio, LC-MS/MS = liquid chromatography with tandem mass spectrometry, NA = not applicable, P_{app} = apparent permeability.

Additional Information: Lemborexant and the metabolites, M4, M9, M10, M13, M14, and M15 were not substrates for BCRP.

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Test Article: lemborexant, M4, M9, M10

Inhibition of P-gp　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Study System	in vitro
Test System	Cultured monolayers of LLC-PK1 cells and human P-gp expressing LLC-PK1 (LLC-MDR1) cells
Method	Incubation of each test article with cell monolayers for 2 hours at 37 °C
Assay/Analyte / Concentration	LSC/^{3}H radioactivity of [^{3}H]digoxin / Lemborexant (0, 2, 5, 10, 20, and 50 µmol/L), and M4, M9, M10 (0, 5, and 20 µmol/L)

Inhibitory Effect of Lemborexant on [^{3}H]Digoxin Transport in P-gp Expressing Cells and Its Control Cells

Marker Substrate	Test Compound	Concentration (µmol/L)	LLC-MDR1			LLC-PK1			CFR (2)	% of Control
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio		
[^{3}H]Digoxin	Control	NA	0.77±0.18	8.35±0.24	10.84	1.93±0.05	5.63±0.06	2.92	7.92	100.0
	Lemborexant	2	0.84±0.19	7.93±0.09	9.44	2.24±0.61	4.87±0.06	2.17	7.27	91.8
		5	0.87±0.13	7.84±0.10	9.01	2.34±0.31	4.54±0.21	1.94	7.07	89.3
		10	1.13±0.13	8.38±0.12	7.42	3.10±0.25	4.47±0.11	1.44	5.98	75.5
		20	1.52±0.12	8.15±0.25	5.36	3.31±0.41	4.13±0.19	1.25	4.11	51.9
		50	2.58±0.03	6.96±0.31	2.70	3.86±0.27	4.05±0.16	1.05	1.65	20.8
	Verapamil	30	1.87±0.23	7.17±0.27	3.83	3.55±0.47	4.19±0.10	1.18	2.65	33.5
[^{14}C]Mannitol		NA	1.94±0.18	1.74±0.35	NA	2.17±0.16	2.36±0.26	NA	NA	NA

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Test Article: lemborexant, M4, M9, M10

Inhibition of P-gp　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Inhibitory Effect of M4, M9, and M10 on [^{3}H]Digoxin Transport in P-gp Expressing Cells and Its Control Cells

Marker Substrate	Test Compound	Concentration (μmol/L)	LLC-MDR1			LLC-PK1			CFR (2)	% of Control
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio		
[^{3}H]Digoxin	Control	NA	0.76±0.05	9.56±0.31	12.58	2.31±0.32	5.75±0.17	2.49	10.09	100.0
	M4	5	0.75±0.06	8.95±0.20	11.93	2.19±0.19	5.29±0.21	2.42	9.51	94.3
		20	0.77±0.11	8.89±0.40	11.55	2.77±0.16	4.77±0.03	1.72	9.83	97.4
	M9	5	0.87±0.06	9.67±0.06	11.11	2.99±0.03	5.13±0.12	1.72	9.39	93.1
		20	0.74±0.04	7.46±0.08	10.08	3.53±0.40	4.21±0.07	1.19	8.89	88.1
	M10	5	0.82±0.04	9.14±0.19	11.15	2.59±0.09	5.06±0.02	1.95	9.20	91.2
		20	1.00±0.10	8.81±0.20	8.81	3.61±0.09	4.14±0.17	1.15	7.66	75.9
	Verapamil	30	2.01±0.15	6.80±0.09	3.38	3.77±0.21	3.95±0.14	1.05	2.33	23.1
[^{14}C]Mannitol		NA	1.99±0.11	2.00±0.27	NA	2.58±0.61	2.08±0.15	NA	NA	NA

Each P_{app} value represents the mean ±SD of 3 samples.　LLC-MDR1:　P-gp expressing cell, LLC-PK1:　control cell.

The [^{3}H]digoxin concentration was 1 μmol/L, and the [^{14}C]mannitol concentration was 1.75 μmol/L.　Verapamil (30 μmol/L) is a typical P-gp inhibitor.

$P_{app\ A\ to\ B}$ = P_{app} value from the apical to the basal side ($\times 10^{-6}$ cm/sec).

$P_{app\ B\ to\ A}$ = P_{app} value from the basal to the apical side ($\times 10^{-6}$ cm/sec).

FR = $P_{app\ B\ to\ A}$ / $P_{app\ A\ to\ B}$.

CFR (2) = FR in LLC-MDR1 − FR in LLC-PK1.

% of control = [CFR (2) in the presence of inhibitor / CFR (2) in the absence of inhibitor] × 100.

CFR = corrected flux ratio, CTD = Common Technical Document, FR = flux ratio, IC_{50} = half-maximal inhibitory concentration, LSC = liquid scintillation counting, NA = not applicable, P_{app} = apparent permeability, P-gp = P-glycoprotein.

Additional Information:　Lemborexant weakly inhibited P-gp dependent [^{3}H]digoxin transport with the IC_{50} values of 20.5 μmol/L.　The IC_{50} values for M4, M9, and M10 on P-gp activities were greater than 20 μmol/L.

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Test Article: lemborexant, M4, M9, M10

Inhibition of P-gp (IC_{50} value)　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Inhibitory Effect of Lemborexant, M4, M9, and M10 on P-gp Transport In Vitro			
Test Compound	**IC_{50} (μmol/L)**	**% of Control at 20 μmol/L[a]**	**% of Control of Verapamil[b]**
Lemborexant	20.5	51.9	33.5
M4	>20	97.4	23.1
M9	>20	88.1	
M10	>20	75.9	

Verapamil (30 μmol/L) is a typical P-gp inhibitor. LLC-MDR1: P-gp expressing cell, LLC-PK1: control cell.

FR = $P_{app\ B\ to\ A}$ / $P_{app\ A\ to\ B}$.

$P_{app\ A\ to\ B}$ = P_{app} value from the apical to the basal side ($\times 10^{-6}$ cm/sec).

$P_{app\ B\ to\ A}$ = P_{app} value from the basal to the apical side ($\times 10^{-6}$ cm/sec).

CFR = corrected flux ratio, CTD = Common Technical Document, FR = flux ratio, IC_{50} = half-maximal inhibitory concentration, P_{app} = apparent permeability, P-gp = P-glycoprotein.

a: % of control = [CFR (2) in the presence of inhibitor / CFR (2) in the absence of inhibitor] × 100. CFR (2) = FR in LLC-MDR1 – FR in LLC-PK1.

b: % of control = [IC_{50} / (IC_{50} + I] × 100, I = concentration of inhibitors.

Additional Information: None.

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Test Article: lemborexant, M4, M9, M10

Inhibition of BCRP　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Study System	in vitro
Test System	Cultured monolayers of MDCK II cells and human BCRP expressing MDCK II (MDCK II-BCRP) cells
Method	Incubation of each test article with cell monolayers for 2 hours at 37 °C
Assay/Analyte / Concentration	LSC/^{3}H radioactivity of [^{3}H]prazosin / Lemborexant (0, 2, 5, 10, 20, and 50 µmol/L), and M4, M9, M10 (0, 5, and 20 µmol/L)

Inhibitory Effect of Lemborexant on [^{3}H]Prazosin Transport in BCRP Expressing Cells and Its Control Cells

Marker Substrate	Test Compound	Concentration (µmol/L)	MDCK II-BCRP			MDCK II			CFR (2)	% of Control
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio		
[^{3}H]Prazosin	Control	NA	1.61±0.20	15.31±0.36	9.51	8.07±0.37	11.59±0.57	1.44	8.07	100.0
	Lemborexant	2	1.77±0.41	15.47±0.38	8.74	9.28±1.01	11.61±0.15	1.25	7.49	92.8
		5	2.15±0.01	17.60±0.34	8.19	10.28±1.27	11.79±0.34	1.15	7.04	87.2
		10	2.87±0.18	18.56±1.13	6.47	12.43±0.18	12.34±0.19	0.99	5.48	67.9
		20	3.46±0.12	18.58±0.64	5.37	14.01±0.37	12.64±0.47	0.90	4.47	55.4
		50	5.18±0.77	18.15±0.83	3.50	14.67±0.96	12.23±0.19	0.83	2.67	33.1
	Ko143	0.05	5.49±0.67	15.21±0.47	2.77	9.48±0.67	11.95±0.63	1.26	1.51	18.7
[^{14}C]Mannitol		NA	1.54±0.16	1.29±0.30	NA	1.33±0.28	1.16±0.22	NA	NA	NA

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Test Article: lemborexant, M4, M9, M10

Inhibition of BCRP　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Inhibitory Effect of Lemborexant on [^{3}H]Prazosin Transport in BCRP Expressing Cells and Its Control Cells

Marker Substrate	Test Compound	Concentration (μmol/L)	MDCK II-BCRP			MDCK II			CFR (2)	% of Control
			$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio	$P_{app\ A\ to\ B}$	$P_{app\ B\ to\ A}$	Flux Ratio		
[^{3}H]Prazosin	Control	NA	1.35±0.06	16.01±0.36	11.86	8.28±0.09	12.35±0.30	1.49	10.37	100.0
	M4	5	1.57±0.19	18.10±0.39	11.53	8.90±0.51	12.68±0.27	1.42	10.11	97.5
		20	2.19±0.27	21.10±2.45	9.63	13.86±0.93	12.83±1.23	0.93	8.70	83.9
	M9	5	1.72±0.08	19.26±0.71	11.20	7.99±0.98	13.81±0.46	1.73	9.47	91.3
		20	2.21±0.15	19.97±0.74	9.04	11.87±0.21	13.81±1.01	1.16	7.88	76.0
	M10	5	2.03±0.06	18.83±0.58	9.28	10.56±0.36	12.67±0.38	1.20	8.08	77.9
		20	2.23±0.10	20.26±1.12	9.09	11.86±0.64	12.27±0.82	1.03	8.06	77.7
	Ko143	0.05	5.36±0.31	14.33±0.60	2.67	9.58±0.66	12.83±0.03	1.34	1.33	12.8
[^{14}C]Mannitol		NA	1.80±0.47	1.50±0.07	NA	1.23±0.05	1.13±0.23	NA	NA	NA

Each P_{app} value represents the mean ±SD of 3 samples.　MDCK II-BCRP:　BCRP expressing cell, MDCK II:　control cell.

The [^{3}H] prazosin concentration was 1 μmol/L, and the [^{14}C]mannitol concentration was 1.75 μmol/L.　Ko143 (0.05 μmol/L) is a typical BCRP inhibitor.

$P_{app\ A\ to\ B}$ = P_{app} value from the apical to the basal side ($\times 10^{-6}$ cm/sec).

$P_{app\ B\ to\ A}$ = P_{app} value from the basal to the apical side ($\times 10^{-6}$ cm/sec).

FR = $P_{app\ B\ to\ A}$ / $P_{app\ A\ to\ B}$.

CFR (2) = FR in MDCK II-BCRP – FR in MDCK II.

% of control = [CFR (2) in the presence of inhibitor / CFR (2) in the absence of inhibitor] × 100.

BCRP = breast cancer resistant protein, CFR = corrected flux ratio, CTD = Common Technical Document, FR = flux ratio, IC_{50} = half-maximal inhibitory concentration, LSC = liquid scintillation counting, NA = not applicable, P_{app} = apparent permeability.

Additional Information:　Lemborexant weakly inhibited BCRP-mediated [^{3}H]prazosin transport with the IC_{50} values of 24.0 μmol/L.　The IC_{50} values of M4, M9, and M10 on BCRP activities were greater than 20 μmol/L.

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Test Article: lemborexant, M4, M9, M10

Inhibition of BCRP (IC_{50} value)　　**Study Number: DMPKT2013-010**　　**Location in CTD: 5.3.2.3.2**

Inhibitory Effect of Lemborexant, M4, M9, and M10 on BCRP Transport In Vitro			
Test Compound	**IC_{50} (µmol/L)**	**% of Control at 20 µmol/L[a]**	**% of Control of Ko143[b]**
Lemborexant	24.0	55.4	18.7
M4	>20	83.9	12.8
M9	>20	76.0	
M10	>20	77.7	

Ko143 (0.05 µmol/L) is a typical BCRP inhibitor. MDCK II-BCRP: BCRP expressing cell, MDCK II: control cell.

CFR (2) = FR in MDCK II-BCRP – FR in MDCK II.

$P_{app\ A\ to\ B}$ = P_{app} value from the apical to the basal side ($\times 10^{-6}$ cm/sec).

$P_{app\ B\ to\ A}$ = P_{app} value from the basal to the apical side ($\times 10^{-6}$ cm/sec).

FR = $P_{app\ B\ to\ A}$ / $P_{app\ A\ to\ B}$.

BCRP = breast cancer resistant protein, CFR = corrected flux ratio, CTD = Common Technical Document, FR = flux ratio, IC_{50} = half-maximal inhibitory concentration, LSC = liquid scintillation counting, NA = not applicable, P_{app} = apparent permeability.

a: % of control = [CFR (2) in the presence of inhibitor / CFR (2) in the absence of inhibitor] × 100.

b: % of control = [IC_{50} / IC_{50} + I] × 100, I = concentration of inhibitors.

Additional Information: None.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: lemborexant, M4, M9, M10

Inhibition of BSEP, OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2-K　　**Study Number: KAC36-02-130827**　　**Location in CTD: 5.3.2.3.3**

Study System	in vitro							
Test System	Vesicular transport inhibition using BSEP expressing vesicles prepared by Sf9 cells. Uptake transporter inhibition using respective transporter expressing cells. CHO cells for OATP1B3, OCT1, OCT2, and OAT1 MDCKII cells for MATE1 and MATE2-K and HEK293 cells for OAT3 and OATP1B1.							
Method	Incubation of lemborexant or its metabolites in the test systems for the designated times at 37 °C							
Assay/Analyte	Transporter inhibition/Respective transporter specific substrates							
Concentration	0.05, 0.16, 0.49, 1.48, 4.44, 13.33, and 40 µmol/L as lemborexant, M4, M9, or M10							
Transporter	**Lemborexant**		**M4**		**M9**		**M10**	
	Maximum inhibition at 40 µmol/L	**IC_{50} (µmol/L)**	**Maximum inhibition at 40 µmol/L**	**IC_{50} (µmol/L)**	**Maximum inhibition at 40 µmol/L**	**IC_{50} (µmol/L)**	**Maximum inhibition at 40 µmol/L**	**IC_{50} (µmol/L)**
BSEP	72%	15.8	22%	ND	18%	ND	34%	ND
MATE1	82%	13.2	27%	ND	59%	32.8	58%	27.6
MATE2-K	74%	12.8	53%	36.9	62%	28.8	49%	ND
OATP1B1	85%	11.1	70%	18.2	72%	17.9	75%	17.3
OATP1B3	82%	14.5	39%	ND	59%	30.5	74%	20.2
OAT1	NIO	ND	NIO	ND	NIO	ND	NIO	ND
OAT3	58%	32.2	33%	ND	34%	ND	36%	ND
OCT1	80%	7.4	73%	9.1	81%	15.0	69%	19.8
OCT2	70%	18.5	67%	20.8	77%	10.1	54%	27.9

ND: IC_{50} could not be calculated as inhibition greater than 50% was not observed within the concentration range tested. NIO: no concentration-dependent interaction observed up to 40 µmol/L.

BSEP = bile salt export pump, CTD = Common Technical Document, HEK = human embryonic kidney, IC_{50} = half-maximal inhibitory concentration, MATE = multidrug and toxin extrusion, ND = not determined, NIO = no interaction observed, OAT = organic anion transporter, OATP = organic anion transporting polypeptide, OCT = organic cation transporter.

Additional Information: Lemborexant showed inhibition, with the exception of OAT1, on all investigated transporters with IC_{50} values of 7.4 to 32.2 µmol/L. With the exception of OCT2, these inhibitory effects of lemborexant were greater than those of its metabolites M4, M9, and M10.

2.6.5.16 薬物動態試験：その他（続き）

Test Article: lemborexant, M4, M9, M10

Transport by OATP1B1 or OATP1B3 **Study Number: KAC37-03-130827** **Location in CTD: 5.3.2.3.4**

Study System	in vitro
Test System	Uptake transporter using respective transporter expressing cells. CHO cells for OATP1B3 and HEK293 cells for OATP1B1.
Method	Incubation of lemborexant or its metabolites in the test systems for 2 or 20 minutes at 37 °C
Assay/Analyte	LC-MS/MS/ Lemborexant, M4, M9, M10
Concentration	0.5, 5 µmol/L as lemborexant, M4, M9, or M10

Calculated Reaction Parameters From OATP1B1 Uptake Transporter Substrate Feasibility Assays With Lemborexant, M4, M9, and M10

Test Article	Concentration (µmol/L)	Incubation Time (min)	Fold Accumulation	
			Without Rifampicin	With Rifampicin
Lemborexant	0.5	2	1.07	0.90
	0.5	20	1.10	0.99
	5	2	1.05	0.87
	5	20	1.30	1.16
M4	0.5	2	1.30	1.01
	0.5	20	1.29	0.64
	5	2	1.20	0.51
	5	20	1.18	0.88
M9	0.5	2	1.24	1.10
	0.5	20	1.17	1.06
	5	2	1.12	0.95
	5	20	1.18	0.78
M10	0.5	2	1.25	0.97
	0.5	20	1.05	1.68
	5	2	1.34	1.64
	5	20	0.82	1.07

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Test Article: lemborexant, M4, M9, M10

Transport by OATP1B1 or OATP1B3 **Study Number: KAC37-03-130827** **Location in CTD: 5.3.2.3.4**

Calculated Reaction Parameters From OATP1B3 Uptake Transporter Substrate Feasibility Assays With Lemborexant, M4, M9, and M10

Test Article	Concentration (µmol/L)	Incubation Time (min)	Fold Accumulation	
			Without Fluvastatin	With Fluvastatin
Lemborexant	0.5	2	1.46	0.82
	0.5	20	1.10	NA
	5	2	1.05	0.93
	5	20	1.31	0.57
M4	0.5	2	0.92	1.27
	0.5	20	1.36	1.50
	5	2	1.46	1.13
	5	20	1.35	1.14
M9	0.5	2	1.22	0.83
	0.5	20	1.62	1.16
	5	2	1.34	1.02
	5	20	1.52	1.26
M10	0.5	2	1.02	0.64
	0.5	20	1.16	0.77
	5	2	0.85	0.70
	5	20	1.37	0.95

Uptake experiments were performed using CHO cells (OATP1B3) or HEK293 cells (OATP1B1) stably expressing the respective uptake transporters. For human OATP1B3, the parental cell lines, and for OATP1B1 mock-transfected HEK293 cells were used as negative control. Rifampicin (50 µmol/L) and fluvastatin (30 µmol/L) were used as reference inhibitor for OATP1B1 and OATP1B3, respectively.

The fold activation value was defined as the ratio of uptake of test article into transfected and control cells:

Fold activation = UPT_{TRP} / $UPT_{Control}$

Where; UPT_{TRP}: accumulated amount of test article in OATP1B1 or OATP1B3 transfected cells normalized by protein content (pmol/mg protein). $UPT_{Control}$: accumulated amount of test article in control cells normalized by protein content (pmol/mg protein).

CTD = Common Technical Document, LC-MS/MS = liquid chromatography with tandem mass spectrometry, OATP = organic anion transporting polypeptide, UPT = uptake.

Additional Information: Lemborexant, M4, M9, and M10 were not substrates of OATP1B1 and OATP1B3.