KMD-3213 (N=10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Specific gravity (g/ml)	1.058	1.066*	1.052	1.064	1.065	1.064	1.066	1.054
Cl- (mEq/l)	179	183	176	192	205	197	181	154
Urine volume (ml/day)	10.5	8.9	11.2	10.2	6.1	8.0	7.6	9.2*
K+ (mEq/l)	370.4	391.1	314.2	387.2	429.1	373.5	384.4	312.6*

Gross pathology: No significant differences were observed between KMD-3213 and MD127K.

MD127K (N=.10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Dark red spot	0	1	1	2	0	0	0	0
Stomach, blood clot on mucosa	0	0	0	0	0	0	0	0
Kidney, recessed focus	1	0	1	0	0	0	0	1

KMD-3213 (N=10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Dark red spot	0	1	2	0	0	0	0	0
Stomach, blood clot on mucosa	0	0	0	1	0	0	0	0
Kidney, recessed focus	1	0	0	0	0	0	0	0

Organ weights: No significant differences were observed between KMD-3213 and MD127K.

MD127K (N=.10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Liver (g)	10.29	10.38	11.02	10.50	7.11	7.39	7.61*	7.61
Spleen (g)	0.46	0.50	0.51	0.53	0.36	0.38	0.37	0.40
Thyroid (g)	0.015	0.014	0.015	0.013	0.013	0.015	0.011	0.011

KMD-3213 (N=10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Liver (g)	10.29	9.98	11.04	11.01	7.11	7.72*	7.56	7.11
Spleen (g)	0.46	0.47	0.53	0.56*	0.36	0.36	0.37	0.35
Thyroid (g)	0.015	0.014	0.015	0.014	0.013	0.013	0.011	0.012

Histopathology: No significant differences were observed between KMD-3213 and MD127K.

MD127K (N=.10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Thyroid gland ultimobranchial remnant	0	ne	ne	1	0	ne	ne	2
Pituitary gland cyst in anterior lobe	0	ne	ne	0	0	ne	ne	1

KMD-3213 (N=10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Thyroid gland ultimobranchial remnant	0	ne	ne	0	0	ne	ne	1
Pituitary gland cyst in anterior lobe	0	ne	ne	1	0	ne	ne	0

Toxicokinetics:

MD127K (N=.10)	Males (mg/kg/day)			Females (mg/kg/day)		
	2	10	50	2	10	50
$AUC_{0\text{-}24hr}$ (nghr/ml)						
__day 1	537.8	2748.2	13138	589.5	2685.1	17281
__week 2	608.2	3520.8	16491	453.3	2077.2	12994
Cmax (5 min.)(ng/ml)						
__day 1	1939.5	9191.7	42108	2163.1	9393.1	59817
__week 2	2118.6	11724	54357	1660.4	7448.3	43743

KMD-3213 (N=10)	Males (mg/kg/day)			Females (mg/kg/day)		
	2	10	50	2	10	50
$AUC_{0\text{-}24hr}$ (nghr/ml)						
__day 1	330.3	2020.5	16012	218.1	1797.6	13730
__week 2	206.8	1923.7	13054	148.5	1538.7	11505
Cmax (5 min.)(ng/ml)						
__day 1	467.5	2000.8	11944	390.7	2093.3	10482
__week 2	402.7	2272.4	10251	307.3	1683.2	10076

Other: No abnormalities were observed in macroscopic observations of the eye, photography of the fundus, or in auditory examinations in either sex for MD127K or KMD-3213.

Study title: KMD-3213: Toxicity to dogs by repeated capsule administration for 52 weeks.

Key study findings:

At 5 mg/kg/day (approximately 2-3 times the expected clinical exposure to silodosin via AUC), pharmacological signs including ptosis were observed, but were reduced in severity by Day 5. Brown discoloration was observed in liver and kidney. Liver tissue stained positive (minimal to slight) for neutral lipids.

20 mg/kg/day (about 12 -19 times the expected clinical exposure via AUC) was a No Observed Adverse Effect Level (NoAEL) in dogs. Although pharmacological signs were observed at this dose, their severity was decreased by Week 3. Brown discoloration was observed in liver and kidney. Liver tissue stained positive (minimal to slight) for neutral lipids. No indication of tissue damage was observed.

At 80 mg/kg/day (about 51 – 118 times), pharmacological signs were observed for the duration of the study, for several hours following administration. Decreased body weights/body weight gain and decreased hemoglobin were observed. Brown discoloration was observed in liver and kidney. Liver tissue stained positive (slight to moderate) for neutral lipids. No indication of tissue damage was observed, but an apparent increase in alkaline phosphatase was observed (without statistical significance at N=4 dogs).

Study no.: KSI 71/974423
Conducting laboratory and location: [redacted]

Date of study initiation: 21 November 1996
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: batch no. HH231, 100.1% pure

Methods

Doses: 0, 5, 20, and 80 mg/kg/day
Species/strain: Beagle dogs
Number/sex/group or time point (main study): 4
Route, formulation, volume, and infusion rate: oral capsules
Toxicokinetics: Blood samples were collected at 2, 8 and 24 hours after administration on Days 1 and Weeks 13 and 26, and at 0.5, 1, 2, 4, 8 and 24hoursafteradministration at Week 52.
Age: 5 months
Weight: 7.4 – 9.9 kg

Results

Mortality: There were no unscheduled deaths.

Clinical signs:

At 5 mg/kg/day, prominent third eyelid, stiff hind leg gait and liquid feces were observed. On Days 1 to 4 of dosing, these signs were generally noted about 1 hour after dosing and were still apparent at the end of the working day, but were resolved by the next day. From Day 5 onwards, the signs were reduced in severity and incidence.

At 20 mg/kg/day, prominent third eyelid, eyes reddened/glazed, squinting, unsteady hind gait, stiff hind leg gait, high stepping gait, trembling and liquid feces were frequently observed. The incidence and severity of the majority of these signs decreased from Week 3 and beyond.

At 80 mg/kg/day, prominent third eyelid, eyes reddened/glazed, unsteady gait, stiff hind leg gait, high stepping gait, trembling, subdued behavior/lying down, liquid feces and vomiting were seen in all animals. These signs were generally noted about one hour after dosing and were still apparent at the end of the working day, but were resolved by the next day. Up to the end of the treatment period the majority of these signs were still being recorded occasionally for a number of animals, but were generally resolved by the end of the working day.

Dose (mg/kg/day)	0 (Control)		5		20		80	
N=	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Clinical signs								
Relaxation of nictitating membrane	–	–	+	+	+	+	+	+
Conjunctival congestion	–	–	–	–	+	+	+	+
Eyelid ptosis	–	–	+	+	+	+	+	+
Decrease in locomotor activity	–	–	–	–	+	+	+	+
Abnormal gait	–	–	+	+	+	+	+	+
Trembling	–	–	–	–	+	+	+	+
Lateral position	–	–	–	–	+	–	+	+
Hunchback position	–	–	–	–	–	–	+	+
Liquid stool	–	–	+	+	+	+	+	+
Vomiting	–	–	+	+	+	+	+	+
Salivation	–	–	–	–	–	–	+	+

Body weights/ food consumption:

Over the period of Weeks 0 - 43, a lower group mean bodyweight gain was noted for males and females receiving 80 mg/kg/day, in comparison with controls, with statistical significance attained by the males. Slightly lower group mean bodyweight gains were also noted for males and females receiving 5 or 20 mg/kg/day.

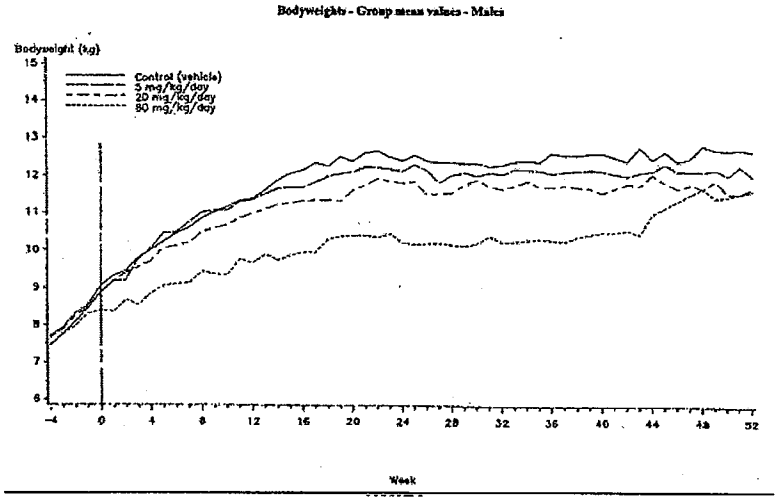
From Week 44, all animals receiving 80 mg/kg/day were offered 500 g diet per day. From this time to Week 52, the mean bodyweight gain of males and females receiving 80 mg/kg/day was statistically significantly higher than that of controls. A statistically significantly higher mean gain was also noted for females receiving 20 mg/kg/day.

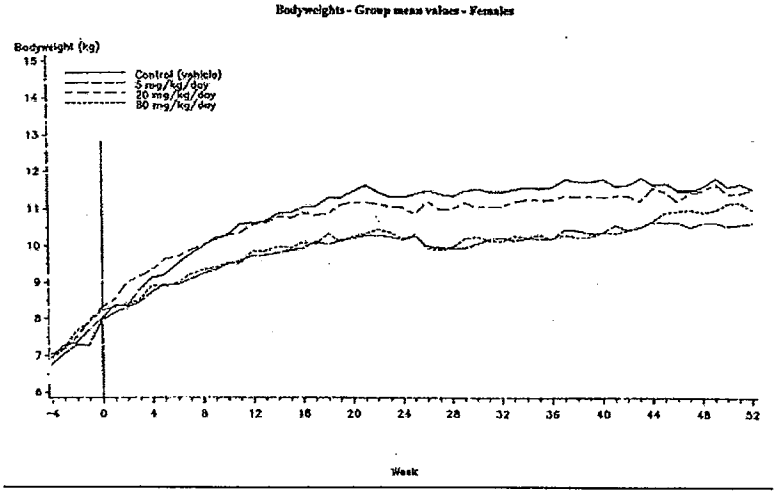
Overall (Weeks 0 - 52) a lower mean gain was noted for males and females receiving 80 mg/kg/day, in comparison with controls.

Marginally lower food intake were recorded for animals of both sexes receiving 80 mg/kg/day compared to controls, during the first 4 weeks of treatment. As the study progressed the effect was less apparent, and group mean values recorded were comparable for both treated and control groups.

Dose (mg/kg/day)	0 (Control)		5		20		80	
N=	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Body weight [a] (kg)	12.7	11.5	12.0	10.6	11.7	11.5	11.6	11.0
Body weight gain (kg)								
Weeks 0 - 43	3.7	3.8	3.2	2.6	2.9	2.9	1.8**	2.2
Weeks 43 - 52	–0.1	–0.3	–0.1	0.1	–0.5	0.3*	1.2**	0.5*
Weeks 0 - 52	3.6	3.5	3.1	2.7	2.8	3.2	2.9	2.6
Food consumption (g/week)								
Week 4	2800	2800	2800	2790	2800	2800	2658	2568
Weeks 1 – 52 [b]	2800	2799	2800	2750	2903	2800	2926	2802
Mean in males and females at Weeks 1 – 52 [b]	2799		2775		2852		2864	

Mean ± S.D., Williams' test (vs control): *; $p<0.05$ **; $p<0.01$. a) At the end of treatment, b) Mean of Week 1 to Week 52.





Ophthalmoscopy:

Ophthalmoscopic examination in Weeks 13, 26, 39 and 52 revealed protrusion of the membrane nictitans and/or blepharospasm from Week 26 in animals receiving 20 or 80 mg/kg/day. As the study progressed these findings were less apparent and their occurrence seemed to be related to the clinical finding of squinting, which also lessened as the study progressed. In some instances the presence of the membrane nictitans made the actual examination of the eye difficult to complete.

All other ophthalmoscopic findings were within normal limits of this age and strain. No changes of toxicological significance were observed.

EKG: No treatment related effects were observed.

Hematology: Minor effects on red blood cell parameters were observed.

Dose (mg/kg/day)		0 (Control)		5		20		80	
N=		M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Hematology									
n=		4	4	4	4	4	4	4	4
RBC ($\times 10^6$/µL)	Week 39	6.36 ± 0.45	6.73 ± 0.26	6.45 ± 0.49	6.94 ± 0.47	6.36 ± 0.44	6.72 ± 0.17	5.67 ± 0.26*	6.26 ± 0.45
	Week 52	6.82 ± 0.59	7.15 ± 0.74	6.80 ± 0.60	6.87 ± 0.48	6.41 ± 0.15[b]	7.17 ± 0.32	6.14 ± 0.51	6.84 ± 0.31
Hemoglobin (g/dL)	Week 39	14.3 ± 1.2	15.3 ± 0.8	14.8 ± 0.9	15.3 ± 1.1	14.1 ± 0.6	14.9 ± 0.6	12.8 ± 0.2*	13.6 ± 0.8*
	Week 52	15.1 ± 1.5	16.0 ± 1.4	15.3 ± 1.0	14.9 ± 1.1	14.2 ± 0.4 [b]	15.6 ± 0.7	13.6 ± 0.6	14.6 ± 0.9
Hematocrit (%)	Week 39	43.5 ± 3.3	46.2 ± 2.8	44.7 ± 3.0	46.6 ± 3.1	42.8 ± 2.0	44.7 ± 1.7	39.1 ± 0.7*	41.5 ± 2.5*
	Week 52	45.8 ± 4.3	48.0 ± 4.0	46.9 ± 2.8	45.6 ± 2.8	43.2 ± 1.0 [b]	47.5 ± 2.2	42.2 ± 2.1	44.8 ± 2.9

Mean ± S.D., Williams' test (vs control): *; p<0.05.

Clinical chemistry: Minor effects on creatinine, urea nitrogen, and calcium were observed at high doses. Apparent increases in alkaline phosphatase at high doses did not reach statistical significance.

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	5	20	80	0	5	20	80
Urea nitrogen (mg/dl)								
__week 13	16	13	15	13	16	15	14*	11**
__week 26	17	17	16	18	18	17	17	13*
__week 39	16	16	16	13	16	14	16	12*
__week 52	18	14	15	12*	18	15	17	11*
Creatinine (mg/dl)								
__week 26	0.8	0.7	0.7	0.8	0.8	0.9	0.8	0.7
__week 39	0.8	0.8	0.7*	0.7**	0.8	0.8	0.8	0.7
__week 52	0.8	0.8	0.7	0.7	0.8	0.8	0.8	0.7
AP (mU/ml)								
__week 26	206	161	166	255	147	161	166	212
__week 39	186	156	140	236	154	174	148	209
__week 52	156	138	142	215	170	175	156	206
Ca (mEq/l)								
__week 13	5.9	5.8	5.8	5.6**	5.9	5.7	5.8	5.6
__week 26	5.4	5.3	5.3	5.1*	5.3	5.3	5.3	5.1
__week 39	5.3	5.4	5.4	5.1	5.3	5.3	5.4	5.0*
__week 52	5.2	5.3	5.4	5.1	5.2	5.2	5.3	5.1
Chloride (mEq/L)								
__week 52	116	116	115	117	114	116	116	118*

Urinalysis: Lower group mean urinary sodium levels were recorded for animals receiving 80 mg/kg/day.

Dose (mg/kg/day)		0 (Control)		5		20		80	
N=		M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Urinalysis									
n=		4	4	4	4	4	4	4	4
Sodium (mEq/vol)	Week 52	15.01 ± 8.40	21.20 ± 17.09	21.77 ± 6.53	22.12 ± 10.14	15.60 ± 13.59	21.71 ± 10.30	8.41 ± 6.32	16.49 ± 7.97

Gross pathology: Dark liver was observed in dogs receiving 20 or 80 mg/kg/day.

Dose (mg/kg/day)	0 (Control)		5		20		80	
N=	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Necropsy findings								
n=	4	4	4	4	4	4	4	4
Liver: Dark coloring	0	0	0	0	1	4	4	4
Gallbladder: Dark color content	0	0	0	0	0	1	1	3

Organ weights: Higher group mean bodyweight-adjusted liver weights were observed for males receiving 20 or 80 mg/kg/day and females receiving 20 mg/kg/day. Bodyweight-adjusted kidney weights were higher and statistically significant for females receiving 80 mg/kg/day, in comparison with the controls.

Dose (mg/kg/day)	0 (Control)		5		20		80	
N=	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4	M: 4	F: 4
Organ weight								
n=	4	4	4	4	4	4	4	4
Absolute organ weight (adjusted for the body weight) (g)								
Liver	424.4 (409.6)	386.0 (381.1)	409.7 (407.0)	379.6 (386.8)	424.1 (436.3)	415.1 (411.7)	475.1 (480.5*)	385.7 (386.8)
Kidney	60.3 (58.0)	47.6 (46.6)	57.7 (57.3)	43.6 (45.2)	56.0 (57.9)	48.6 (47.9)	58.0 (58.9)	53.0 (53.2**)
Relative organ weight (g/100 g)								
Liver	3.43	3.47	3.47	3.68	3.77	3.73	4.11	3.57
Kidney	0.49	0.43	0.49	0.42	0.50	0.44	0.51	0.49

Mean ± S.D. or Mean.
Williams' test (vs control): *; p<0.05 **; p<0.01.

Histopathology: Adequate Battery: yes (x), no ( )—explain
Peer review: yes ( ), no (x)

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	5	20	80	0	5	20	80
Lungs								
__congestion, minimal	0	0	0	1	0	0	0	0
__subpleural fibrosis and inflammatory cells	1	0	1	3	0	1	0	1
_____minimal	0	0	0	1	0	1	0	0
_____slight	1	0	1	2	0	0	0	1
Liver								
__portal fibrosis and mononuclear cells, slight	0	0	0	1	0	0	0	1
__fine brown pigment deposits in hepat.(mainly centr), min.	1	2	2	3	2	0	3	3
__subcaps fibrosis, bile duct prolif, pigmented macr., slight	0	0	0	1	0	0	0	0
__bile duct proliferation, fibrosis and inflammation	0	0	1	0	0	0	1	0
____minimal	0	0	0	0	0	0	1	0
____slight	0	0	1	0	0	0	0	0
__liver cell necrosis, inflammation and fibrosis, mod.	0	0	1	0	0	0	0	0
__hepatocyte hypertrophy, minimal	0	0	0	0	0	0	1	0
__Brown pigment in Kupffer cells, minimal	2	3	3	3	3	4	3	3
__tiss. neg. stain.: Schmorl's, Alcian Blue, & Perls' stains	1	0	0	0	1	0	0	0
__tiss. neg. stain.: Schmorl's & Alcian Blue stains	0	0	0	1	0	0	0	0
__lipofuscin-like pigment and positive Smorl's stain	0	0	0	0	0	0	0	1
__tissue negative staining with Alcian Blue	0	0	0	0	0	0	0	1
Liver with ORO (neutral lipid) stain								
__yellow/brown pigment deposits in hepat. (mainly centr.)	0	3	4	4	0	3	4	4
____minimal	0	1	3	0	0	3	2	0
____slight	0	2	1	3	0	0	2	3
____moderate	0	0	0	1	0	0	0	1
__lipofuscin-like pigment & positive Smorl's and Sudan Black	0	0	0	1	0	0	0	1
Gall bladder, aggregates of lymphocytes	0	2	2	2	1	0	1	1
Kidney, brown pigment deposits in cortical tubules	1	3	4	4	2	1	4	4
__minimal	1	1	2	1	2	1	2	3
__slight	0	1	2	3	0	0	2	1
__moderate	0	1	0	0	0	0	0	0

Toxicokinetics:

Blood samples were taken on Day 1 and during Weeks 13, 26 and 52 in order to assess the systemic exposure of male and female dogs to KMD-3213 following daily oral (capsule) administration of KMD-3213 at dose levels of 5, 20 and 80 m mg/kg/day. Samples were taken at 2, 8 and 24 hours post-dose on Day 1 and during Weeks 13 and 26, and at 0.5, 1, 2, 4, 8 and 24 hours post-dose during Week 52. Plasma concentrations of KMD-3213 were measured by a validated high performance liquid chromatographic (HPLC) method.

Mean maximum plasma concentrations (Cmax) of KMD-3213 and the mean areas under the plasma KMD-3213 concentration-time curves estimated up to 24 hours post-dose (AUC_{24}) on Day 1 and during Weeks 13, 26 and 52, derived from the reduced sampling schedule, are presented below with standard deviations in parentheses:

Dose level	C_{max} (ng/ml)							
	Day 1		Week 13		Week 26		Week 52	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
5	370.4	275.0	374.7	267.7	270.2	178.9	229.4	129.6
	(158.5)	(111.0)	(113.0)	(56.2)	(116.6)	(72.4)	(210.7)	(47.1)
20	1504.8	1401.4	2154.1	1317.0	2021.6	1347.9	1489.3	1017.6
	(262.0)	(459.9)	(521.5)	(717.2)	(390.3)	(532.8)	(164.2)	(659.7)
80	4794.1	4131.7	4553.2	4901.9	4527.3	5000.9	2972.6	7237.1
	(722.6)	(1172.0)	(478.0)	(2751.6)	(1978.8)	(584.1)	(1582.0)	(1213.7)

Dose level	AUC_{24} (ng.h/ml)							
	Day 1		Week 13		Week 26		Week 52	
(mg/kg/day)	Males	Females	Males	Females	Males	Females	Males	Females
5	1513	1128	1582	1253	1274	1009	1082	582
	(672)	(436)	(498)	(266)	(606)	(233)	(1091)	(250)
20	6420	6143	9798	5991	9802	6129	7301	4628
	(1139)	(1814)	(2235)	(3384)	(1418)	(2586)	(591)	(3181)
80	27513	23517	25755	26643	28838	30824	19147	44097
	(8643)	(14086)	(4461)	(16533)	(9125)	(5130)	(9096)	(8534)

Tmax was at about 2 hours.

Plasma concentrations of KMD-3213 at 24 hours post-dose (C24) were not quantifiable in all animals on all sampling days, except for females at the 80 mg/kg/day dose level where C24 was quantifiable on all sampling days, therefore, these animals were continuously exposed to KMD-3213 during a dosing interval.

Mean maximum plasma concentrations (Cmax) of KMD-3213 and the mean areas under the plasma KMD-3213 concentration-time curves estimated up to 24 hours post-dose (AUC_{24}) during Week 52, derived from the full sampling schedule, are presented below with standard deviations in parentheses:

Dose level (mg/kg/day)	C_{max} (ng/ml) Males	C_{max} (ng/ml) Females	AUC_{24} (ng.h/ml) Males	AUC_{24} (ng.h/ml) Females
5	364.4 (118.1)	611.0 (528.7)	1034 (795)	894 (490)
20	2723.1 (440.4)	2465.8 (1248.4)	8335 (528)	5472 (3494)
80	4668.9 (3497.3)	9146.5 (1475.0)	22081 (10942)	43004 (4829)

Study title: 13-Week Oral Dose Study in Dogs

Key study findings: A No Observed Adverse Effect Level (NoAEL) for delayed maturation of testes and epididymis and absence of sperm was 10 mg/kg/day (about 5 times the expected clinical exposure). At 50 mg/kg/day (about 64 times), these effects were observed; however, they were not apparent at termination of the 80 mg/kg/day group in the 52 week study.

Study no.: KSI 70/970908

Methods

Doses: 0, 10, 50, and 100/200 mg/kg/day (Reduced to 100 mg/kg/day at the end of Week 1)

Species/strain: Beagle dogs

Number/sex/group or time point (main study): 3

Route, formulation, volume, and infusion rate: oral, gelatin capsule

Toxicokinetics: Blood samples were collected at 0.5, 1, 2, 4, 8 and 24 hours after administration.

Age: 20-25 weeks

Results

Dose (mg/kg/day)		0 (Control)		10		50		100/200 [a]	
N=		M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Toxicokinetics									
AUC_{0-24} (ng * hr/mL)	Day 1	Not done	Not done	1788 ± 689	2493 ± 1073	23705 ± 7096	23293 ± 2896	69492 ± 34565	41581 ± 22630
	Week 13	Not done	Not done	3350 ± 962	3063 ± 1156	30096 ± 11116	25627 ± 6153	39327 [b]	38975 ± 9150
C_{max} (ng/mL)	Day 1	Not done	Not done	544.3 ± 420.8	822.7 ± 384.4	4126.7 ± 1514.0	5128.0 ± 1004.7	5931.6 ± 1936.2	8728.8 ± 2631.8
	Week 13	Not done	Not done	1017.2 ± 319.3	995.7 ± 227.6	4813.1 ± 1746.0	5918.5 ± 2868.9	7698.6 [b]	5961.2 ± 1862.2
T_{max} (hr)	Day 1	Not done	Not done	2.2 ± 1.8	1.2 ± 0.8	2.3 ± 1.5	1.3 ± 0.6	2.0 ± 1.7	1.0 ± 0
	Week 13	Not done	Not done	1.7 ± 0.6	1.7 ± 0.6	1.3 ± 0.6	1.2 ± 0.8	1.0 [b]	1.3 ± 0.6

Mean ± S.D., Williams' test (vs. control): *; $p<0.05$ **; $p<0.01$.

a) The test article was administered initially at 200 mg/kg, which was decreased to 100 mg/kg on Day 7.

b) 1animal.

Effects on body weight gain were observed at 50 mg/kg/day. Clinical signs were observed at all treated doses.

Dose (mg/kg/day)	0 (Control)		10		50		100/200 a)	
N=	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Noticeable findings								
Number of animals died or sacrificed moribund	0	0	0	0	0	0	1	0
Body weight b) (kg)	11.7	10.6	12.8	11.4	10.5	10.3	10.0	10.1
Body weight gain (Weeks 0 – 13) (kg)	1.9	2.1	2.3	2.0	1.1	1.3	−0.1*	1.1*
Food consumption c) (g/week)	2800	2799	2800	2800	2800	2800	2591	2714
Mean food consumption in males and females c) (g/week)	2800		2800		2800		2659**	
Clinical signs								
Relaxation of nictitating membrane	–	–	+	+	+	+	+	+
Conjunctival congestion	–	–	+	+	+	+	+	+
Eyelid ptosis	–	–	+	+	+	+	+	+
Decrease in or loss of locomotor activity	–	–	–	+	–	+	+	+
Trembling	–	–	–	+	+	+	+	+
Liquid stool	–	+	+	+	+	+	+	+
Vomiting	–	–	–	+	+	+	+	+
Salivation	–	–	–	–	–	+	+	+
Abnormal gait (ataxic gait)	–	–	–	–	+	+	+	+
Lateral position	–	–	–	–	–	+	+	+

Mean, Williams' test (vs control): *; p<0.05 **; p<0.01.

-: No noticeable finding, +: Finding noted.

a) The test article was administered initially at 200 mg/kg, which was decreased to 100 mg/kg on Day 7.

b) At the end of treatment

c) Mean during the treatment period.

Dose (mg/kg/day)	0 (Control)		10		50		100/200 a)	
N=	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Ophthalmological examination	–	–	–	–	–	–	–	–
Electrocardiogram b)	–	–	–	–	–	–	–	–
Heart rate b) (beats/min)	133 ± 37.5	118 ± 42.7	109 ± 13.2	121 ± 28.1	107 ± 8.1	116 ± 25.5	86 ± 14.8	114 ± 13.0
Hematology	–	–	–	–	–	–	–	–
Blood chemistry=								
n=	3	3	3	3	3	3	2	3
Triglyceride (mg/dL) Week 13	44 ± 5.0	42 ± 4.0	45 ± 13.5	34 ± 1.7	28 ± 9.1	32 ± 7.0*	35 ± 0.7	24 ± 5.2**
Total protein (g/dL) Week 13	5.6 ± 0.26	5.6 ± 0.26	5.6 ± 0.26	5.4 ± 0.21	5.4 ± 0.12	5.2 ± 0.06	5.2 ± 0.35	5.1 ± 0.32*
Albumin (g/dL) Week 13	2.8 ± 0.10	3.0 ± 0.21	2.8 ± 0.35	2.9 ± 0.15	2.5 ± 0.31	2.5± 0.15*	2.6 ± 0.07	2.7 ± 0.15*
Urinalysis	–	–	–	–	–	–	–	–
Bone marrow cytology	–	–	–	–	–	–	–	–
Organ weight								
n=	3	3	3	3	3	3	2	3
Absolute organ weight (g)								
Thymus:	10.0	10.3	10.8	11.5	4.5	8.0	5.5	5.7*
Relative organ weight (g/100 g)								
Thymus:	0.09	0.10	0.09	0.10	0.04	0.08	0.05	0.06
Necropsy findings								
n=	3	3	3	3	3	3	2	3
Thymus: Small-sized	0	0	0	0	2	0	1	1

Mean ± S.D., Williams' test (vs control): *; p<0.05 **; p<0.01.

-: No noticeable finding, +: Finding noted.

a) The test article was administered initially at 200 mg/kg, which was decreased to 100 mg/kg on Day 7.

b) Examined 24 hours after administration at the end of treatment period.

At 50 mg/kg/day, delayed maturation of testes and epididymis and absence of sperm were observed.

Dose (mg/kg/day)	0 (Control)		10		50		100/200 a)	
N=	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3	M: 3	F: 3
Histopathology								
n=	3	3	3	3	3	3	2	3
Thymus: Atrophy (minor)	2	1	1	1	0	1	1	3
Atrophy (mild to severe)	0	0	0	0	2	0	1	0
Prostate: Delayed maturation	1		1		3		2	
Epididymis: Sperm absent	0		0		2		1	
Testis: Delayed maturity	0		0		2		1	

Mean ± S.D.

a) The test article was administered initially at 200 mg/kg, which was decreased to 100 mg/kg on Day 7.

Study Title: Two-week oral toxicity study of KMD-3213 in Beagle dogs.

Study no.: 70158
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano 399-8305, Japan
Date of study initiation: 17 May 1994
GLP compliance: no
QA report: yes ( ) no (x)
Drug, lot #, and % purity: KMD-3213, lot #OZA-862
Methods

Doses: 50, 200, and 500 mg/kg/day
Species/strain: Beagle dog
Number/sex/group or time point (main study): 2 males/group
Route, formulation, volume, and infusion rate: intravenous
Age: 6 months
Weight: 6.7-7.8 kg

Results:

Mortality: One dog in the high dose group died on day 8.

Clinical signs:

50 mg/kg/day	200 mg/kg/day	500 mg/kg/day
Vomiting of food, vomiting of test substance, mucus stool	Vomiting of food, vomiting of test substance, vomiting of foamy substance, mucus stool, prolapse of haw, loose stool, diarrhea	Vomiting of food, vomiting of test substance, vomiting of foamy substance, vomiting of bloody substance, mucus stool, loose stool, diarrhea, tarry stool, decreased spont. activity, prolapse of haw, watery stool, bloody stool, death

Body weights:

50 mg/kg/day	200 mg/kg/day	500 mg/kg/day
	Slight decrease	Slight decrease

Food consumption: A slight decrease in food consumption was observed in the dog that died on day 7.

EKG:

50 mg/kg/day	200 mg/kg/day	500 mg/kg/day
	QT prolongation, decrease in heart rate, sinus bradycardia	QT prolongation, decrease in heart rate, sinus bradycardia

Hematology: No treatment related effects were observed.
Clinical chemistry: No treatment related effects were observed.
Urinalysis: No treatment related effects were observed.

Histopathology:

50 mg/kg/day	200 mg/kg/day	500 mg/kg/day
Liver: granulomatous lesion; Kidney: focal basophilic changes in tubules	Kidney: focal basophilic changes in tubules	Liver: vacuolization in cytoplasm of centrilobular hepatocytes

histopathology inventory

Study	KSI 114/012990	KSI 100/012988	KSI 102/012989
Species	Mouse, male	Mouse, female	Rat
Adrenals	X	X	X
Aorta	X	X	X
Bone Marrow smear			
Bone (femur)	X	X	X
Brain	X*	X*	X
Cecum	X	X	X
Cervix			X
Coagulating glands			
Colon	X	X	X
Duodenum	X	X	X
Epididymis	X*		X
Esophagus	X	X	X
Eye	X	X	X
Fallopian tube			
Gall bladder	X	X	
Gross lesions			
Harderian gland	X	X	X
Heart	X*	X*	X*
Ileum	X	X	X
Injection site			
Jejunum	X	X	X
Kidneys	X*	X*	X
Lachrymal gland	X	X	X
Larynx			
Liver	X*	X*	X*
Lungs	X*	X*	X*
Lymph nodes, cervical			
Lymph nodes mandibular	X	X	X
Lymph nodes, mesenteric	X	X	X
Mammary Gland	X	X	X
Optic nerves	X	X	X
Ovaries		X*	X*
Pancreas	X	X	X
Parathyroid	X	X	X
Peripheral nerve			
Pharynx			
Pituitary	X*	X*	X*
Prostate	X*		X*
Rectum	X	X	X
Salivary gland	X*	X*	X*
Sciatic nerve	X	X	X
Seminal vesicles	X*		X*
Skeletal muscle	X	X	X
Skin			
Spinal cord	X	X	X
Spleen	X*	X*	X*
Sternum	X	X	X
Stomach	X	X	X
Testes	X*		X*
Thymus	X*	X*	X*
Thyroid	X	X	X*
Tongue	X		X
Trachea	X		X
Urinary bladder	X	X	X
Uterus		X*	X*
Vagina		X	X
Zymbal gland			X
Standard List			

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

Study title: Reverse mutation test of KMD-3213 using bacteria

Key findings: KMD-3213 did not increase the number of revertant colonies at any dose tested and was judged to be not mutagenic under the conditions tested.

Study no.: 10036

Conducting laboratory and location: Toxicology Research Laboratory, R&D Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 3 August 1995
GLP compliance: Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: lot number GD231, 99.6% pure

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA100, TA1535, TA98, and TA1537 and *Escherichia coli* strain WP2uvrA

Doses used in definitive study: 3000, 1500, 750, 374, 187.5, 93.8, and 46.9 pg/plate in the presence and absence of rat hepatic microsomal drug metabolism enzymes (S9-mix)

Basis of dose selection: A dose-finding study was performed. Using 5000 pg/plate as the highest dose level, 1000, 500, 100, 50, and 10 pg/plate of KMD-3213 were tested in duplicate for the toxicity on the test strains. No background bacterial growth occurred at the KMD-3213 level of 5000 pg/plate in both cultures with or without metabolic activation Therefore, 3000 pg/plate was used as the highest dose for the definitive study

Negative controls: DMSO

Positive controls: 2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide, Sodium azide, N-Ethyl-N'-nitro-N-nitrosoguanidine, 9-Aminoacridine hydrochloride, and 2-Aminoanthracene

Incubation and sampling times: The preincubation method was used in the presence and absence of S9. The sampling time was 48 hours.

Results

Study validity : Two independent tests were conducted on triplicate samples. Positive and negative controls performed as expected.

Study outcome: KMD-3213 did not increase the number of revertant colonies at any dose tested and was judged to be not mutagenic under the conditions tested.

Study title: Reverse mutation test of MD127K in bacteria

Key findings: MD127K, the glucuronide metabolite of KMD-3213, was found to be not mutagenic under the conditions of this study.

Study no.: 10292

Submission #046, Volume #2, and page #533
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano, 399-8305, Japan
Date of study initiation: 19 May 2003
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MD127K, Lot #124-001-26-01, 99.04% pure

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA100, TA98, TA1535, and TA1537 and *Escherichia coli* strain WP2*uvrA*/pKM101

Doses used in definitive study: 0, 156, 313, 625, 1250, 2500, and 5000 ug/plate without S9 and 0, 78, 156, 313, 625, 1250, and 2500 ug/plate with S9.

Basis of dose selection: bacterial growth inhibition observed at $\geq$ 2500 ug/plate with metabolic activation

Negative controls: DMSO

Positive controls: AF-2 (in TA100 at 0.01 ug/plate, Ta98 at 0.1 ug/plate, and *E. coli* at 0.005 ug/plate), sodium azide (in TA1535 at 0.5 ug/plate), 9-aminoacridine (in TA1537 at 80 ug/plate), 2-aminoanthracene (+S9 in all strains)

Incubation and sampling times: preincubation method, with and without metabolic activation (S9 from phenobarbital and 5,6-benzoflavone induced rat liver) at 48 hours

Results

Study validity: Positive and negative controls responded as expected.

Study outcome: No increase of 2-fold or greater revertant colonies were observed at any concentration of MD127K, which was judged to be not mutagenic under the conditions of this assay.

Study title: Chromosomal aberration test of MD127K in cultured Chinese hamster cells.

Key findings: MD127K, the glucuronide metabolite of KMD-3213, was found to be not mutagenic under the conditions of this assay.

Study no.: 10298

Submission #046, Volume #2, and page #563
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano, 399-8305, Japan
Date of study initiation: 19 May 2003
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: MD127K, lot # 056-021119-1, 00.00% pure

Methods

Strains/species/cell line: Chinese hamster lung fibroblast cell

Doses used in definitive study: 0, 1250, 2500, and 5000 ug/ml

Basis of dose selection: No limiting toxicity was observed at these doses in a range finding study.

Negative controls: 0.5% methylcellulose solution

Positive controls: mitomycin C (), cyclophosphamide ()

Incubation and sampling times: 6 hours ± S9 and 24 hours –S9 (from phenobarbital and 5,6-benzoflavone induced rat liver

Results

Study validity: Positive and negative controls responded as expected.

Study outcome: No increase in the frequency of cells with structural chromosomal aberrations or polyploid cells was observed at any concentration of MD127K, which was judged to be not mutagenic under the conditions of this assay.

Study title: Mammalian cell mutation assay

Key findings: Increases in mutant frequency were not observed at any dose tested in either the absence or the presence of S-9, and it was concluded that KMD-3213 was not genotoxic under the conditions tested.

Study no.: KSI 80/973223

Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 17 June 1997
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: silodosin, batch number JH312, 100.3% pure

Methods

Strains/species/cell line: mouse lymphoma L5178Y cells

Doses used in definitive study:

Mutation tests: -S-9 mix Test 1: 5, 15, 30, 60, 125, 250, 375, 500 µg/ml; Test 2: 50, 100, 150, 200, 250, 300, 350, 400 µg/ml

Mutation tests: +S-9 mix Test 1: 5, 15, 30, 60, 125, 200, 250, 300 µg/ml; Test 2: 50, 100, 150, 200, 250, 300, 350, 400 µg/ml

Basis of dose selection:

Preliminary toxicity test: 30, 75, 150, 300, 600, 1125, 2250, 3000 µg/ml, using 80-90 % reduction of plating efficiency as a criteria for toxicity.

Negative controls: DMSO

Positive controls: Methyl methanesulphonate (- S9) and 20-Methylcholanthrene (+ S9)

Incubation and sampling times: The incubation time was 3 hours. Mutant frequency was assessed after 48 hours.

Results

Study validity: Two independent tests were performed, each in the absence and the presence of S9.

Study outcome: Increases in mutant frequency were not observed at any dose tested in either the absence or the presence of S-9, and it was concluded that KMD-3213 was not genotoxic under the conditions tested.

Study title: Chromosomal aberration test of KMD-3213 with mammalian cells in culture

Key findings: No increase in chromosomal aberrations was observed at any dose silodosin tested by the 24- or 48-hour direct method or by the 6-hour treatment activation method in the presence of S-9. However, in the 6-hour treatment in the absence of S-9, chromosomal aberrations were observed and were confirmed in an additional assay. Although mitotic index was not measured in this study, an additional study was also performed (see 7L425 below), in which decreased mitotic index (toxicity) was found to be associated with chromosomal aberrations under similar conditions at similar concentrations.

b(4)

Study no.: 2626 (005-013)

Conducting laboratory and location:

Date of study initiation: 23 June 1995
GLP compliance: yes, Japanese MHW
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot # GD231, 99.6% pure

Methods

Strains/species/cell line: Chinese hamster lung fibroblast cells (CHL)

Doses used in definitive study:

Based on the results of the cell growth inhibition test, the following 5 doses were selected for chromosome aberration test.

Treatment	Doses of KMD-3213 (µg/ml)				
24-hr treatment by the direct method	21.9,	43.8,	87.5,	175,	350
48-hr treatment by the direct method	21.9,	43.8,	87.5,	175,	350
Metabolic activation method with S9-mix	87.5,	175,	350,	700,	1400
Metabolic activation method without S9-mix	87.5,	175,	350,	700,	1400

The cells treated at the underlined doses were investigated for chromosome aberration.

For the confirmatory test by the metabolic activation method without S9-mix, 5 doses of 50.0, 200, 350, 500, and 650 ug/ml were selected.

Basis of dose selection: The doses were selected based on the results of a cell growth inhibition test.

Negative controls: 0.5% methylcellulose solution

Positive controls: mitomycin C (0.05 ug/ml) and cyclophosphamide (12.5 ug/ml)(in the presence of metabolic activation)

Incubation and sampling times:

"Direct method"

CHL cells were suspended in the medium to make a concentration of 8 x 10^3 cells/ml for the 24-hour treatment and 4 x 10^3 cells/ml for the 48-hour treatment. One milliliter of each cell fluid was inoculated into each well of a multi-plate for cell culture (24-well plate; [redacted]). On day 3 of the culture, 100 ul of the test solution or the solvent was added and cultured for a further 24 or 48 hours. Cell survival rate was determined as a ratio of spectrophotometric absorbance to that of the solvent control. b(4)

"Metabolic activation method"

Cell suspensions were prepared with the medium so as to contain 8 x 10^3 cells/ml, and 1 ml of the cell suspension was inoculated into each well of multi-plate. On day 3 of culture, 500 ul of the medium was removed, and 100 ul of S9-mix and 60 ul of the test solution or the solvent was added in the activation group with S9-mix. In the activation group without S9-mix, 400 ul of the medium was removed, and 60 ul of the test solution alone or the solvent alone was added (without S9-mix). After 6 hours, the medium was removed from each well and the cells were washed with isotonic saline. Then, 500 ul of fresh medium was added and the cells were cultured for a further 18 hours. The cell survival rate was then determined.

Calculation of the 50% cell growth inhibition concentration

The medium was removed from the wells of each plate, and the cells were washed once with saline. After neutral buffered formalin solution for tissue fixation [redacted], Lot No. D1006) was added to fix the cells for about 10 minutes, the cells were stained with 0.1% crystal violet solution [redacted], Lot No. 607E4067) for 10 minutes. Each plate was washed with water and then dried well. b(4)

An appropriate amount of pigment eluate (30% ethanol, 1% aqueous acetic acid solution) was added to each well. After standing for about 5 minutes, absorbance was measured at the wavelength of 580 nm, using a spectrophotometer (Model [redacted], [redacted] The cell survival rate in each treatment group was calculated as a ratio of b(4)

the amount of eluted pigment to that in the solvent control group, and the 50% cell growth inhibition concentration of the test solution was calculated by the probit method.

For this calculation, the data at 8 doses in the range of 13.4 - 480 ug/ml were used for the 24- and 48-hr treatment by the direct method, the data at 5 doses ranging from 22.4 to 173 ug/ml were for the treatment by the activation method with S9-mix, and the data at 5 doses ranging from 104 to 800 pg/ml were for the treatment by the activation direct method without S9-mix.

Results

Study validity: A hundred metaphase cells per plate were observed. 2 plates were used for each dose. Cell survival was measured using crystal violet; mitotic index was not measured. The positive and negative control groups responded as expected. The incidence of aberrant cells was determined for the cases including and excluding the cells with gaps alone (+gap and -gap). Final evaluation was based on the number of aberrant cells including the cells with gaps alone. When 2 or more types of structural aberration appeared in the same cell, each type of aberration was counted as one aberration.

The following criteria were used for evaluation of the incidences of cells with structural aberrations and polyploid cells. When the result was reproducible and dose-dependent, the test article was judged to be positive for induction of chromosome aberration.

No statistical analysis was employed for evaluation of data.

< 5%;	negative (-)
5%, <10%;	false positive (±)
10%;	positive (+)

Study outcome:

The KMD-3213 concentrations required to inhibit cell growth in 50% of the cells observed were as follows:

24-hour treatment by the direct method: 132 ug/ml

48-hour treatment by the direct method: 51.7 ug/ml

Treatment by the activation method with S9-mix: 661 ug/ml

Treatment by the activation method without S9-mix: 390 ug/ml

No increase in chromosomal aberrations was observed at any dose KMD-3213 tested by the 24- or 48-hour direct method or by the 6-hour treatment activation method in the presence of S-9. However, in the 6-hour treatment in the absence of S-9, the following results were observed and confirmed in an additional assay. Although mitotic index was

not measured in this study, an additional study (7L425) was also performed, in which decreased mitotic index was found to be associated with chromosomal aberrations under similar conditions.

Results of the chromosome aberration test on CHL cells treated with KMD-3213 (activation method: minus S9)

Compound	Dose (μg/ml)	Number of cells	No. of cells with structural aberrations						Total (+gap) (%)	Total (-gap) (%)	Polyploid cells (%)	Final judgement
			gap	ctb	cte	csb	cse	oth				
0.5% MC a)	0	200	1	2	0	0	0	0	1.5 −	1.0 −	0.5 −	−
KMD-3213	87.5	200	3	3	3	0	0	0	4.5 −	3.0 −	0.0 −	−
	175	200	1	5	2	0	0	0	3.0 −	3.0 −	0.5 −	−
	350	200	3	5	10	0	0	0	7.5 ±	6.5 ±	1.0 −	±
	700	Toxic										
CP b)	12.5	200	0	0	2	0	0	0	1.0 −	1.0 −	0.5 −	−

ctb: Chromatid break cte: Chromatid exchange csb: Chromosome break cse: Chromosome exchange oth: others
a): Solvent control
b): Positive control

Results of the confirmative examination of KKD-3213 [activation method: minus S9)

Compound	Dose (μg/ml)	Number of cells	No. of cells with structural aberrations						Total (+gap) (%)	Total (-gap) (%)	Polyploid cells (%)	Final judgement
			gap	ctb	cte	csb	cse	oth				
0.5%MC a)	0	200	0	2	1	0	0	0	1.5 −	1.5 −	0.0 −	−
KMD-3213	50.0	200	1	3	0	0	0	0	2.0 −	1.5 −	1.5 −	−
	200	200	2	2	1	0	0	0	1.5 −	1.0 −	0.0 −	−
	350	200	1	1	3	0	1	0	3.0 −	2.5 −	0.5 −	−
	500	200	4	12	15	0	0	0	13.5 +	12.0 +	0.0 −	+
	650	Toxic										
CP b)	12.5	200	1	4	2	0	0	0	3.0 −	3.0 −	1.0 −	−

ctb: Chromatid break cte: Chromatid exchange csb: Chromosome break cse: Chromosome exchange oth: others
a): Solvent control
b): Positive control

Study title: Chromosomal aberration test of KMD-3213 with mammalian cells in culture (by short-term treatment without chromosomal activation)

Key findings: Decreased mitotic index (toxicity) was found to be associated with chromosomal aberrations at the highest concentration of silodosin tested.

Study no.: 7L425

Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 28 July1997
GLP compliance: Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot No. JH312, 100.9% pure)

Methods

Strains/species/cell line: Chinese hamster lung

Doses used in definitive study: 0, 37.5, 75, 150, 300, and 600 ug/ml

Basis of dose selection: 1000 ug/ml was 100% lethal under the conditions tested.

Negative controls: cell medium

Positive controls: MMC, 15 ug/ml

Incubation and sampling times:

Five milliliters of a 4 x 10^3 cell/ml cell suspension was inoculated into each Petri dish, 6 cm in diameter, and incubated for 3 days.

After the culture medium was removed from the Petri dish, the cells were treated with 0.3 ml of the test substance suspension and 2.7 ml of culture medium for 6 hours; then the cells were washed 3 times with MEM; and further incubated in 5 ml of a fresh culture medium for 18 hours.

The suspending medium used for preparation of the test substance suspensions (0.5% methylcellulose solution in water) was likewise treated as the negative control. Two Petri dishes were used for each concentration.

Results

Study validity: Two plates were used for each concentration. The cells in metaphase in 1000 cells per plate and those in 2000 cells per test substance concentration were counted to calculate the mitotic indices. Positive and negative controls responded as expected.

Study outcome:

Mitotic index (short-term treatment without metabolic activation)

Treatment	Addition of S9 mix	Treating concentration (μg/ml)	Number of cells observed	Number of cells in metaphase	Mitotic index (%)	Mitotic activity (%)
Negative control (MC)	-	0	2000	125	6.3	100
KMD-3213	-	100	2000	135	6.8	108
	-	200	2000	119	6.0	95
	-	400	2000	94	4.7	75
	-	600	2000	61	3.1	49
	-	800	2000	31	1.6	25
	-	1000	1119	10	0.89	14

MC: 0.5% solution of methylcellulose in water

Mitotic activity: Ratio of mitotic index for each treated group to the negative control

Results of chromosomal aberration test (short-term treatment without metabolic activation)

Treatment	Addition of S9 mix	Treating concentration (μg/ml)	Number of cells observed	Number and Incidence of diploid cells (%)	Reading 2)	Number and Incidence (%) of cells with aberrations in chromosome structure 1)								
						Gap	Chromatid		Chromosome		fg	Total		Reading 2)
						gap	ctb	cte	csb	cse		-gap	+gap	
Solvent (methylcellulose)	-	0	100	0	-	0	2	0	0	0	0	2	2	-
			100	0		0	0	0	0	0	0	0	0	
			200	0 (0.0)		0 (0.0)	2 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)	2 (1.0)	
Test substance	-	37.5	100	0	-	0	1	0	0	0	0	1	1	-
			100	0		0	1	1	0	1	0	3	3	
			200	0 (0.0)		0 (0.0)	2 (1.0)	1 (0.5)	0 (0.0)	1 (0.5)	0 (0.0)	4 (2.0)	4 (2.0)	
	-	75 ◇	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		0	0	1	0	0	0	1	1	
			200	0 (0.0)		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	
	-	150 ◆	100	0	-	0	2	1	0	0	0	3	3	-
			100	1		0	0	0	0	0	0	0	0	
			200	1 (0.5)		0 (0.0)	2 (1.0)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.5)	3 (1.5)	
	-	300 ◆	100	0	-	0	0	0	0	0	0	0	0	-
			100	0		0	1	1	0	0	0	1	1	
			200	0 (0.0)		0 (0.0)	1 (0.5)	1 (0.5)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)	1 (0.5)	
	-	600 ◆	100	0	-	1	9	12	0	0	0	17	17	+
			88	0		0	11	16	1	0	0	22	22	
			188	0 (0.0)		1 (0.5)	20 (10.6)	28 (14.9)	1 (0.5)	0 (0.0)	0 (0.0)	39 (20.7)	39 (20.7)	
Positive control (MMC)	-	0.15	100	0	-	1	18	41	0	0	0	54	54	+
			100	0		0	7	44	0	0	0	47	47	
			200	0 (0.0)		1 (0.5)	25 (12.5)	85 (42.5)	0 (0.0)	0 (0.0)	0 (0.0)	101 (50.5)	101 (50.5)	

1) Gap: Gap of chromatids or chromosomes; ctb: chromatid break; cte: chromatid exchange; csb: chromosome break; cse: chromosome exchange; fg: fragmentation

2) The test was read as negative (-) if the incidence of cells with + gap aberrations in chromosome structure or that of cells with aberrations in chromosome number was less than 5%; doubtful positive (±) if the incidence was not less than 5% and less than 10%; and positive (+) if the incidence was not less than 10%.

Treatment time with the test substance: 6 hours; the cell recovery time after treatment: 18 hours.

Methylcellulose: 0.5% solution of methylcellulose in water; MMC: mitomycin C

◇: A precipitate of the test substance was found in the culture medium.

◆: A precipitate and a float of the test substance were found in the culture medium.

Study title: Micronucleus study of KMD-3213 in mice

Key findings: No increase in micronuclei was observed at any dose KMD-3213 tested, and KMD-3213 was judged to be not genotoxic under the conditions of this assay.

Study no.: 10067

Conducting laboratory and location: Toxicology Research Laboratory, RRD Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano Pref., 399-8305 Japan

Date of study initiation: 17 June 1996
GLP compliance: Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot no. GD231, 99.6% pure

Methods

Strains/species/cell line: Mouse, ICR (CD-1), SPF, male, 8 weeks old, 32.1 – 38.0 g

Doses used in definitive study: 0, 250, 500, and 1000 mg/kg (N=6), oral

Basis of dose selection: Based on the results of a preliminary study in which 2000 mg/kg was lethal, the highest dose for the present study was set at 1,000 mg/kg.

Negative controls: 0.5% methylcellulose solution

Positive controls: Mitomycin C, 1 mg/kg ip

Incubation and sampling times: Bone marrow specimens were prepared 24 hours following treatment.

Results

Study validity: For each animal, 2000 polychromatic erythrocytes were counted. 1000 whole erythrocytes per animal were counted to obtain the ratio of reticulocytes to erythrocytes. Positive and negative controls responded as expected.

Study outcome: No increase in micronuclei was observed at any dose KMD-3213 tested. The ratio of reticulocytes to erythrocytes was similar in all KMD-3213 dose groups, in the negative control group, and in the positive control group (1 mg/kg MMC). One treatment related death occurred at 500 mg/kg.

Study title: Rat liver DNA repair (UDS) test

Key findings: KMD-3213 did not cause any significant increases in either the gross nuclear grain count or the net nuclear grain count (i.e. the gross nuclear pain count minus the cytoplasmic grain count) at any dose level at either sampling time, and was therefore judged to be not genotoxic under the conditions of this assay.

Study no.: KSI 083/974372

Volume #, and page #:
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 27 October 1997
GLP compliance: yes, OECD
QA reports: yes (x) no ( )
Drug, lot #, and % purity: batch no. JH312, 100.3% pure

Methods

Strains/species/cell line: rat, male Sprague-Dawley, Hsd/Ola

Doses used in definitive study: 0, 600, and 2000 mg/kg, (N=5)

Basis of dose selection: Treatment related deaths were observed at 2000 mg/kg in a preliminary study.

Negative controls: vehicle (aqueous 0.5% (w/v) methyl cellulose)

Positive controls: dimethylnitrosamine (4 mg/kg) (2 hour expression) or 2-acetylaminofluorene (50 mg/kg) (14 hour expression)

Incubation and sampling times:

Hepatocytes were isolated by enzymatic dissociation at 2 or 14 hours after exposure of the animals to the test substance. Four animals were assessed at each experimental point (two animals from the positive control group). Due to the death of two animals prior to the end of the 14 hour expression time at 2000 mg/kg, only three animals were assessed at this experimental point.

The isolated hepatocytes were allowed to attach to glass coverslips and were cultured in vitro with (methyl-^{3}H) thymidine at 10 uCi/ml for four hours to radiolabel replicating DNA. The hepatocytes were chased for 24 hours with unlabelled thymidine, fixed and processed for autoradiography.

Results

Study validity: DNA repair was assessed by comparing the labeling levels of hepatocyte nuclei from treated animals with control values and with the accompanying cytoplasmic labeling levels (usually a total of 150 cells per animal were examined). Positive control group animals showed a large statistically significant increase in the net nuclear grain count which was accompanied by a large increase in the gross nuclear grain count.

Study outcome: KMD-3213 did not cause any significant increases in either the gross nuclear grain count or the net nuclear grain count (i.e. the gross nuclear pain count minus the cytoplasmic grain count) at any dose level at either sampling time, and was therefore judged to be not genotoxic under the conditions of this assay.

Results for the 2 hour expression

Treatment	Dosage (mg/kg)	Gross nuclear grain count x1	x2	x3	Mean	Group mean†	Cytoplasmic grain count x1	x2	x3	Mean	Group mean	Net nuclear grain count x1	x2	x3	Mean	Group mean †
Vehicle	-	13.7	11.6	15.0	13.4	12.3	14.3	15.3	18.7	16.1	15.2	-0.5	-3.7	-3.7	-2.6	-2.9
		8.8	9.8	10.6	9.7		12.9	12.6	13.9	13.1		-4.1	-2.8	-3.3	-3.4	
		12.0	12.1	13.4	12.5		15.8	12.9	14.2	14.3		-3.8	-0.8	-0.8	-1.8	
		13.0	14.8	13.0	13.6		15.5	19.4	17.0	17.3		-2.5	-4.5	-4.0	-3.7	
KMD-3213	600	12.9	14.7	15.7	14.4	12.7 ns	17.0	17.5	20.4	18.3	16.9	-4.1	-2.8	-4.7	-3.9	-4.2 ns
		13.3	14.1	13.6	13.7		18.7	18.9	17.9	18.5		-5.3	-4.8	-4.2	-4.8	
		12.4	9.6	11.3	11.1		15.3	14.2	14.4	14.6		-2.9	-4.6	-3.1	-3.5	
		11.3	11.4	12.0	11.6		15.9	15.9	17.1	16.3		-4.6	-4.5	-5.1	-4.7	
KMD-3213	2000	11.4	12.0	13.5	12.3	12.4 ns	16.9	16.6	18.5	17.3	16.7	-5.4	-4.6	-5.0	-5.0	-4.3 ns
		12.4	12.9	11.5	12.3		17.1	15.9	15.6	16.2		-4.7	-3.0	-4.1	-3.9	
		11.4	14.5	13.9	13.3		18.0	17.8	17.8	17.9		-6.6	-3.3	-3.9	-4.6	
		11.1	12.3	12.2	11.9		14.5	15.0	16.9	15.5		-3.4	-2.7	-4.7	-3.6	
DMN	4	30.5	29.1	34.8	31.5	32.0 **	12.9	13.5	15.2	13.9	12.6	17.6	15.6	19.5	17.6	19.4 **
		36.8	29.4	31.1	32.4		11.4	11.9	10.4	11.2		25.3	17.5	20.7	21.2	

DMN Dimethylnitrosamine
x1, x2, x3 Mean results for each replicate culture
† Results of statistical analysis (one-way analysis of variance followed by a Student's *t* test with critical one-sided probability levels):
** P < 0.001 (highly significant)
ns P > 0.01 (not significant)
NB An apparent discrepancy of 0.1 grains can occasionally occur due to rounding of mean values. Net grain count = Gross minus Cytoplasmic count.

Results for the 14 hour expression

Treatment	Dosage (mg/kg)	Gross nuclear grain count x1	x2	x3	Mean	Group mean†	Cytoplasmic grain count x1	x2	x3	Mean	Group mean	Net nuclear grain count x1	x2	x3	Mean	Group mean †
Vehicle	-	11.4	9.6	10.7	10.6	10.2	15.6	12.6	14.0	14.1	13.4	-4.1	-3.0	-3.3	-3.5	-3.3
		9.2	12.1	9.4	10.2		13.8	14.3	11.2	13.1		-4.6	-2.2	-1.9	-2.9	
		10.4	10.0	10.9	10.4		14.3	12.5	14.7	13.8		-3.9	-2.5	-3.8	-3.4	
		6.7	10.6	11.0	9.4		10.0	13.7	14.4	12.7		-3.2	-3.1	-3.5	-3.3	
KMD-3213	600	10.8	11.0	10.5	10.8	11.0 ns	14.3	14.5	14.8	14.5	14.6	-3.5	-3.5	-4.3	-3.8	-3.5 ns
		11.6	9.8	10.9	10.8		14.4	14.5	15.0	14.6		-2.7	-4.7	-4.1	-3.8	
		12.0	11.8	11.4	11.7		16.3	14.1	13.7	14.7		-4.3	-2.3	-2.3	-3.0	
		10.1	11.0	11.6	10.9		13.9	14.2	15.2	14.4		-3.8	-3.2	-3.5	-3.5	
KMD-3213	2000	12.1	10.7	10.8	11.2	10.8 ns	17.5	15.1	13.6	15.4	15.1	-5.4	-4.4	-2.8	-4.2	-4.4 ns
		8.9	9.7	8.3	9.0		11.7	12.3	12.4	12.1		-2.8	-2.6	-4.1	-3.2	
		13.1	10.8	12.5	12.1		18.9	16.2	18.4	17.8		-5.9	-5.4	-6.0	-5.8	
		ND	ND	ND	ND		ND	ND	ND	ND		ND	ND	ND	ND	
AAF	50	32.2	28.4	30.6	30.4	27.9 **	15.0	12.7	11.0	12.9	11.8	17.2	15.8	19.5	17.5	16.1 **
		24.0	26.4	25.5	25.3		12.4	9.4	10.2	10.7		11.7	17.0	15.4	14.7	

AAF 2-Acetylaminofluorene
x1, x2, x3 Mean results for each replicate culture
† Results of statistical analysis (one-way analysis of variance followed by a Student's *t* test with critical one-sided probability levels):
** P < 0.001 (highly significant)
ns P > 0.01 (not significant)
NB An apparent discrepancy of 0.1 grains can occasionally occur due to rounding of mean values. Net grain count = Gross minus Cytoplasmic count.
ND No data available due to death of animal prior to end of expression time.

2.6.6.5 Carcinogenicity

Study title: KMD-3213: Carcinogenicity Study by Dietary Administration to CD-1 Mice for 104 Weeks

Key study findings: Mammary gland adenoacanthomas , mammary gland adenocarcinomas, and mammary gland adenomas or carcinomas were statistically significant and were considered drug related.

Study number: KSI 100/012988
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 14 May 1999
GLP compliance: yes
QA report: yes (x) no ( )
Drug: lot #JN301, 100.2% pure and JR261, 100.2% pure b(4)

Study Type: 2 year bioassay
Species/strain — CD-1 (ICR) BR mice
Number/sex/group; age at start of study: 50 females/group/ 6 weeks/ 21.5-31.2 g
Animal housing: 2/cage
Formulation/vehicle: dietary
Drug stability/homogeneity: checked weeks 1, 13, 26, 39, 52, 65, 78, 91, and 102
Methods:
Doses: 0, 0, 60, 150, 400 mg/kg/day
Basis of dose selection: AUC ratio
Route of administration: oral, dietary
Dual controls employed: yes
Interim sacrifices: no
Satellite PK or special study group(s): 32 per dose level

Results:

Mortality:

	Females (mg/kg/day)				
	0	0	60	150	400
Weeks 1 to 102*	38	23	25	32	34
Main group mortality (%)	76	46	50	64	68
Main group survival (%)	24	54	50	36	32

* In addition, 1 group 1 control, 1 group 2 control and 1 60 mg/kg/day female were found dead during the period of the terminal kill.

Clinical signs: No treatment related clinical signs were observed

Body weights: There was an overall lower body weight gain, from approximately week 10 in the 400 mg/kg/day group compared with combined controls (-24% at week 102). The corresponding body weight gains for the 60 and 150 mg/kg/day groups were –5% and –14 %, respectively.

	Females (mg/kg/day)				
	0	0	60	150	400
Week 26, weight (g)	41.4	43.3	40.3	41.4	39.1
Week 102, weight (g)	46.0	44.3	42.6	44.5	39.9
Week 102, weight gain (g)	20.8	19.2	16.9	18.8	15.0*
Week 102, % of combined controls	--	--	86	95	76

Food consumption: No treatment related effects were observed for food consumption or food conversion efficiency.

Hematology: No treatment related effects were observed.

Clinical chemistry: Not measured.

Organ weights: Increases in pituitary and uterine weights and relative liver weight were observed at 150 mg/kg/day and above.

	Females (mg/kg/day)				
	0	0	60	150	400
Terminal body weight (g)	47.2	44.5	42.4	44.6	40.5
Pituitary (g)	.0032	.0037	.0032	.0043*	.0116**
Uterus + cervix (g)	2.354	0.804	0.796	0.473*	0.287**
Liver (g) (adjusted for body weight)	1.957	2.126	2.214	2.712**	2.888**

Gross pathology:

<102 weeks	Females (mg/kg/day)				
Number of animals with palpable swellings	11	5	11	16	21
Pituitary (N)	39	24	26	32	34
__swollen	0	0	0	1	2
__mass(es)	0	1	1	1	4
Uterus (N)	39	24	26	32	34
__mass(es)	4	2	1	2	1
__thickened	14	6	6	2	7
__fluid swelling(s)	18	15	6	7	9
__cyst(s)	1	1	4	5	4
Subcutis (N)	39	24	26	32	34
__mass present	2	2	2	7	12
__second mass present	0	0	0	0	1
Mammary	39	24	26	32	34
__thickening	0	0	0	0	4
Liver (N)	39	24	26	32	34
__pale	19	14	13	18	23

102 weeks	Females (mg/kg/day)				
Pituitary (N)	11	26	24	18	16
__swollen	0	0	0	3	2
__mass(es)	0	1	0	0	6
Uterus (N)	11	26	24	18	16
__mass(es)	1	8	3	2	1
__thickened	3	15	13	2	1
__fluid swelling(s)	7	19	14	9	6
__cyst(s)	1	2	10	8	6
Subcutis (N)	11	26	24	18	16
__mass present	1	1	1	4	3
__second mass present	0	0	0	0	2
Mammary gland	11	26	24	18	16
__thickening	0	0	0	0	0

Liver	11	26	24	18	16
__pale	0	2	3	4	4

All animals (N=50)	Females (mg/kg/day)				
Pituitary (N)					
__swollen	0	0	0	4	4
__mass(es)	0	2	1	1	10
Uterus (N)					
__mass(es)	5	10	4	4	2
__thickened	17	21	19	4	8
__fluid swelling(s)	25	34	20	16	15
__cyst(s)	2	3	14	13	10
Subcutis (N)					
__mass present	3	3	3	11	15
__second mass present	0	0	0	0	3
Mammary gland					
__thickening	0	0	0	0	4
Liver					
__pale	19	16	16	22	27

Histopathology:

Non-neoplastic:

<102 weeks	Females (mg/kg/day)				
	0	0	60	150	400
Mammary glands (N)	39	24	26	32	34
__lymphoid infiltration	2	0	1	1	8
__lobular hyperplasia	1	1	3	7	21
__acinar dilatation	9	6	3	9	14
__atypical hyperplasia	0	0	0	3	5
__squamous metaplasia	0	0	0	3	2
__ductular dilatation	1	0	1	3	6
Pituitary (N)	36	24	24	29	34
__hypertrophy-pars distalis, focal	1	0	0	0	7
__hyperplasia-pars distalis, diffuse	0	0	0	2	9
__hyperplasia-pars distalis, focal	0	1	0	1	4
Sciatic (N)	37	24	26	32	34
__degenerate fibres	13	5	14	19	25
Thyroids (N)	39	24	25	32	34
__follicular dilatation	6	4	5	4	11
Uterus (N)	39	24	26	32	34
__cystic endometrial hyperplasia	12	7	6	2	3
__cystic glands	2	2	1	2	9
__adenomyosis	7	4	13	20	16
Liver (N)	39	24	26	32	34
__parenchymal inflammatory cell foci	3	2	3	5	6
__leukocytosis	2	3	3	2	5
__single cell necrosis	0	0	1	1	2
Mesenteric lymph node	39	24	26	32	34
__inflammatory cell infiltration	1	4	4	6	9
__increased cellularity, generalized	2	3	3	3	5
__sinus erythrocytosis/erythrophagatosis	6	8	8	9	19
__hemosiderosis	2	6	6	5	16
__sinus histiocytosis	0	0	1	2	1

__vascular mural fibrinoid necrosis	0	0	0	0	2
__extramedullary hematopoiesis	3	1	4	3	11
Lymphoid infiltration/aggregates/ or foci					
__kidneys	8	7	6	9	19
__pancreas	2	2	1	2	7
__skeletal muscle	2	0	2	2	5
__salivary glands	17	12	12	16	21
__thyroids	0	0	2	1	1
__urinary bladder	12	12	8	12	18

102 weeks	Females (mg/kg/day)				
	0	0	60	150	400
Mammary glands (N)	11	26	24	18	16
__lymphoid infiltration	0	0	0	1	4
__lobular hyperplasia	1	3	2	8	15
__acinar dilatation	1	1	0	4	6
__atypical hyperplasia	0	1	1	3	7
__squamous metaplasia	0	0	1	3	3
__ductular dilatation	1	2	1	3	8
Pituitary	11	26	21	13	16
__hypertrophy-pars distalis, focal	2	2	0	3	3
__hyperplasia-pars distalis, diffuse	0	0	0	0	3
__hyperplasia-pars distalis, focal	1	0	0	2	3
Sciatic (N)	11	26	24	18	16
__degenerate fibres	8	22	24	18	16
Thyroids (N)	11	26	0	0	16
__follicular dilatation	4	5	0	0	6
Uterus (N)	11	26	24	18	16
__cystic endometrial hyperplasia	6	16	14	1	1
__cystic glands	1	0	3	8	1
__adenomyosis	4	3	15	14	12
Liver (N)	11	26	24	18	16
__parenchymal inflammatory cell foci	5	9	8	16	16
__leukocytosis	0	0	3	0	5
__single cell necrosis	0	1	0	2	7
Mesenteric lymph node (N)	11	26	24	18	16
__inflammatory cell infiltration	1	2	2	8	4
__increased cellularity, generalized	1	9	4	1	3
__sinus erythrocytosis/erythrophagatosis	6	3	6	10	11
__hemosiderosis	5	5	7	13	9
__sinus histiocytosis	1	1	1	0	0
__extramedullary hematopoiesis	1	1	1	5	5
Lymphoid infiltration (N)	11	26	0	0	16
__thyroids	0	3	0	0	6

Total of all animals	Females (mg/kg/day)				
	0	0	60	150	400
Mammary glands (N)	50	50	50	50	50
__lymphoid infiltration, total	2	0	1	2	12**
_____minimal	1	0	1	1	12
_____ slight	1	0	0	0	0
_____ moderate	0	0	0	1	0
__lobular hyperplasia, total	2	4	5	15***	36***
_____minimal	1	4	3	11	17
_____ slight	1	0	1	4	10

_____ moderate	0	0	1	0	6
_____ marked	0	0	0	0	3
__acinar dilatation, total	10	7	3	13	20*
_____ minimal	10	7	3	10	12
_____ slight	0	0	0	2	5
_____ moderate	0	0	0	1	2
_____ marked	0	0	0	0	1
__atypical hyperplasia, total	0	1	1	6*	12***
_____ minimal	0	0	1	4	10
_____ slight	0	0	0	1	2
_____ moderate	0	1	0	0	0
_____ marked	0	0	0	1	0
__squamous metaplasia, total	0	0	1	6*	5
_____ minimal	0	0	1	5	5
_____ moderate	0	0	0	1	0
__ductular dilatation, total	2	2	2	6	14**
_____ minimal	1	2	1	6	8
_____ slight	1	0	1	0	3
_____ moderate	0	0	0	0	3
Pituitary (N)	47	50	45	42	50
__hypertrophy-pars distalis, focal, total	0	0	0	2	12***
_____ minimal	0	0	0	2	6
_____ slight	0	0	0	0	4
_____ moderate	0	0	0	0	2
__hyperplasia-pars distalis, diffuse, total	1	1	0	3	7
_____ minimal	1	1	0	3	4
_____ slight	0	0	0	0	1
_____ moderate	0	0	0	0	2
__hyperplasia-pars distalis, focal, total	3	2	0	3	10
_____ minimal	3	2	0	2	9
_____ slight	0	0	0	1	0
_____ moderate	0	0	0	0	1
Sciatic nerve (N)	48	50	50	50	50
__degenerate fibres, total	21	27	38**	37**	41***
_____ minimal	13	11	20	18	22
_____ slight	7	11	11	15	11
_____ moderate	1	4	7	3	7
_____ marked	0	1	0	1	1
Uterus (N)	50	50	50	50	50
__cystic endometrial hyperplasia, total	18	23	20	3***	4**
_____ minimal	5	5	7	2	3
_____ slight	4	8	8	1	0
_____ moderate	6	9	2	0	1
_____ marked	3	1	3	0	0
__cystic glands, total	3	2	4	10	10
_____ minimal	3	0	1	2	5
_____ slight	0	2	2	7	3
_____ moderate	0	0	1	1	1
_____ marked	0	0	0	0	1
__adenomyosis, total	11	7	28***	34***	28***
_____ minimal	8	4	15	18	19
_____ slight	2	2	6	9	5
_____ moderate	1	1	7	3	3
_____ marked	0	0	0	4	1
__glandular dilatation, total	2	9	7	5	12**

____minimal	1	5	5	3	6
____slight	1	4	2	2	6
Liver (N)	50	50	50	50	50
__parenchymal inflammatory cell foci, total	8	11	11	21**	22**
____minimal	8	11	9	17	18
____slight	0	0	2	4	3
____moderate	0	0	0	0	1
__leukocytosis, total	2	3	6	2	10*
__single cell necrosis, total	0	1	1	3	9**
____minimal	0	1	1	3	9
Mesenteric lymph node (N)	50	50	50	50	50
__inflammatory cell infiltration, total	2	6	6	14**	13**
____minimal	2	6	6	12	11
____slight	0	0	0	2	1
____moderate	0	0	0	0	1
__sinus erythrocytosis/erythrophagatosis, total	12	11	14	19	30***
____minimal	10	9	11	16	21
____slight	1	2	3	2	8
____moderate	1	0	0	1	0
____marked	0	0	0	0	1
__hemosiderosis, total	7	11	13	18*	25***
____minimal	6	11	11	13	19
____slight	1	0	2	5	6
__extramedullary hematopoiesis, total	4	2	5	8	16**
____minimal	4	2	5	7	14
____slight	0	0	0	1	2
Kidneys (N)	50	50	26	32	50
__perivascular lymphoid aggregations, total	14	20	6	9	32**
____minimal	14	20	6	8	26
____slight	0	0	0	0	4
____moderate	0	0	0	0	2
____marked	0	0	0	1	0
__peripelvic lymphoid aggregations, total	3	13	5	5	18**
____minimal	3	13	5	4	18
____slight	0	0	0	1	0
Pancreas (N)	50	50	26	32	50
__interstitial lymphoid foci, total	3	9	1	2	13*
____minimal	3	6	1	2	13
____slight	0	3	0	0	0
Skeletal muscle (N)	50	50	26	32	50
__lymphoid aggregates, total	3	1	2	2	8
____minimal	3	1	1	2	7
____slight	0	0	1	0	1
Salivary glands (N)	50	50	26	32	50
__interstitial lymphoid aggregates, total	23	27	12	16	34*
____minimal	19	24	9	16	30
____slight	3	3	2	0	4
____moderate	1	0	1	0	0
Thyroids (N)	50	50	25	32	50
Lymphoid infiltration, total	0	3	2	1	7*
____minimal	0	3	1	1	7
____moderate	0	0	1	0	0

*Fisher's Exact test on totals only, compared with group 1 ($p<0.005$)
**Fisher's Exact test on totals only, compared with group 1 ($p<0.01$)
***Fisher's Exact test on totals only, compared with group 1 ($p<0.001$)

Neoplastic:

<102 weeks	Females (mg/kg/day)				
	0	0	60	150	400
Mammary glands	39	24	26	32	34
__M-mammary adenocarcinoma	3	1	1	6	9
__M-mammary adenoacanthoma	1	1	0	0	6
__M-carcino-sarcoma	0	0	0	0	1
Pituitary	36	24	24	29	34
__B-adenoma, pars distalis	0	0	1	2	2
__M-carcinoma, pars distalis	0	0	0	0	1
Liver (N)	39	24	26	32	34
__B-hepatocellular adenoma	3	2	1	4	4
__M-hepatocellular carcinoma	0	0	0	1	2

102 weeks	Females (mg/kg/day)				
	0	0	60	150	400
Mammary glands	11	26	24	18	16
__M-mammary adenocarcinoma	1	1	1	1	3
__M-mammary adenoacanthoma	0	0	0	1	5
__B-mammary adenoma	0	1	0	1	0
Pituitary	11	26	21	13	16
__B-adenoma, pars distalis	1	2	0	1	3
Liver (N)	11	26	24	18	16
__B-hepatocellular adenoma	0	5	3	3	6
__M-hepatocellular carcinoma	0	1	0	1	0

Total of all animals	Females (mg/kg/day)				
	0	0	60	150	400
Mammary glands					
__M-mammary adenocarcinoma	4	2	2	7	12
__M-mammary adenoacanthoma	1	1	0	1	11
__carcino-sarcoma	0	0	0	0	1
__adenoma	0	1	0	1	0
Pituitary					
__B-adenoma, pars distalis	1	2	1	3	5
__M-carcinoma, pars distalis	0	0	0	0	1
Liver (N)					
__B-hepatocellular adenoma	3	7	4	7	10
__M-hepatocellular carcinoma	0	1	0	2	2

Background data in CD-1 female mice from studies conducted January 1992 to June 1999											
Study code	A	B	C	D	E	F	G	H	I	J	K
Carcinoma, pars distalis	0	0	0	0	0	0	0	0	1	0	0
Adenoma, pars distalis	0	1	1	0	2	1	1	3	0	3	0
Number of pituitaries examined	54	54	60	50	50	55	47	50	50	60	50
Adenocarcinoma	2	0	1	2	2	1	3	2	0	3	1
Adenoacanthoma	2	0	0	0	0	1	0	0	0	1	0
Number of mammary glands examined	56	56	60	50	50	56	50	50	47	60	69
Hepatocellular carcinoma	0	0	0	0	0	0	0	1	1	0	0
Hepatocellular adenoma	2	1	0	3	3	0	3	0	0	2	1
Number of livers examined	56	56	60	50	50	56	50	50	50	60	69

Factors contributory to death before 102 weeks	Females (mg/kg/day)				
	0	0	60	150	400
Mammary adenocarcinoma	3	1	1	5	8
Histiocytic sarcoma	1	1	1	4	2
Mammary adenoacanthoma	0	1	0	0	3
Pituitary tumor	0	0	0	0	3

Toxicokinetics: (AUC in humans at 4mg dose is ~200 nghr/ml)

Week 26	Females (mg/kg/day)		
	60	150	400
Cmax (ng/ml)	168.04	1476.31	4189.61
Cmin (ng/ml)	41.20	249.70	531.96
AUC_{24} (nghr/ml)	2543	16298	64058

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The doses were adequate based on multiples of parent drug and metabolites for this non-genotoxic drug. All CAC and division suggestions were incorporated in final study. CAC concurred that the study was adequate.

Evaluation of tumor findings: Mammary gland adenoacanthomas, mammary gland adenocarcinomas, and mammary gland adenomas or carcinomas were statistically significant and were considered drug related.

Study title: KMD-3213: Carcinogenicity Study by Dietary Administration to Male CD-1 Mice for 104 Weeks (replacement study for male mice killed in excessive numbers through fighting during the previous 2-year assay)

Key study findings: The study was negative for treatment related neoplasms

Study number: KSI 114/012990
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 20 August 1999
GLP compliance: yes
QA report: yes (x) no ( )
Drug, lot #, and % purity: batches JN301 (100.2% pure), JR261 (100.2% pure), JZ011 (100.5% pure)

b(4)

Study Type: 2 year bioassay
Species/strain: [redacted] CD-1 (ICR) BR mice
Number/sex/group; age at start of study: 50/group, ~ 6 weeks of age, 23-34 g
Animal housing: individual cages
Formulation/vehicle: dietary
Drug stability/homogeneity: confirmed on weeks 1, 13, 26, 39, 52, 78, 91, and 103

Methods:

Doses: 0, 0, 20, 60, and 200/100 mg/kg/day (due to substantially reduced body weight gain, doses reduced in the high dose group from week 27 of treatment)
Basis of dose selection: AUC ratios and MTD
Restriction paradigm for dietary restriction studies: none
Route of administration: dietary
Dual controls employed: yes
Interim sacrifices: no
Satellite PK or special study group(s): 32 per dose level

Results:

Mortality:

	Males (mg/kg/day)				
	0	0	20	60	200/100
Weeks 1 to 104	32	24	27	26	24
Main group mortality (%)	64	48	54	52	48
Main group survival (%)	36	52	46	48	52

Clinical signs: No treatment related effects were observed.

Body weights:

	Males (mg/kg/day)				
	0	0	60	150	400
Week 26, weight (g)	50.2	51.3	49.9	47.9	43.7
Week 104, weight (g)	49.6	49.3	49.4	49.5	48.3
Weeks 0-26, weight gain (g)	21.6	22.5	--	--	14.6**
Weeks 26-104, weight gain (g)	20.7	20.6	20.3	19.9	19.2
Weeks 0- 104, weight gain (g)	20.7	20.6	20.3	19.9	19.2
Week 104, % of combined controls	--	--	99	97	93

Food consumption:

	Males (mg/kg/day)				
	0	0	60	150	400
Week 26 (g/animal)	45	43	45	41	40
Week 52 (g/animal)	43	45	43	40	38
Week 78 (g/animal)	43	45	46	41	42
Week 104 (g/animal)	41	41	37	36	36
Weeks 1-104, % of combined controls	--	--	102	92	92

Hematology: No treatment related effects were observed.

Clinical chemistry: not measured

Organ weights:

(organ weights adjusted for body weight)	Males (mg/kg/day)				
	0	0	20	60	200/100
Terminal body weights (g)	49.1	48.7	49.0	49.0	48.4
Epididymides (g)	0.117	0.121	0.124	0.130*	0.129*
Heart (g)	0.286	0.285	0.257**	0.256**	0.253**
Prostate (g)	0.091	0.091	0.080	0.076	0.077*
Seminal vesicles (g)	1.654	1.625	2.287	1.900	1.711
Liver (g)	3.026	3.160	2.546	2.632	2.797

Gross pathology:

Died or were sacrificed before 104 weeks	Males (mg/kg/day)				
	0	0	20	60	200/100
Liver, enlarged	1/32	2/24	5/27	3/26	7/24
Coagulating gland (N)	32	24	27	26	24
__distended	8	9	12	14	15
__mass(es)					1
__congested			1		
Lymph node, lumbar, regional to mass			1	1	3
Seminal vesicles, distended	12/32	8/24	16/27	16/26	14/24

Termination at 104 weeks	Males (mg/kg/day)				
Liver, enlarged	0	0	0	1	0
Testes, pale area(s)	0/18	1/26	0/23	2/24	3/26
Coagulating gland (N)	18	26	23	24	26
__distended	7	7	19	22	19
__mass(es)			1		
Lymph node, lumbar, regional to mass			1		1
Penis/prepuce, protruding				1	2
Seminal vesicles, distended	13/18	18/26	21/23	19/24	17/26

All animals (N=50)	Males (mg/kg/day)				
Liver, enlarged	1	2	5	4	7
Seminal vesicles, distended	25	26	37	35	31
Coagulating gland					
__distended	15	16	31	36	34
__mass(es)			1		1
__congested			1		
Lymph node, lumbar, regional to mass			2	1	4
Seminal vesicles, distended	25	26	37	35	31

Histopathology:

Non-neoplastic:

Died or were sacrificed before 104 weeks	Males (mg/kg/day)				
	0	0	20	60	200/100
Coagulating gland (N)	32	23	27	26	24
__distended	11	13	23	24	23
__interstitial lymphoid infiltration	1	1	6	10	7
__interstitial inflammation	1	0	5	3	1
__interstitial fibrosis	0	0	1	1	2
Seminal vesicle (N)	32	24	27	26	24
__distension	11	8	17	20	18
__interstitial lymphoid infiltration	0	0	2	1	3
__interstitial fibrosis	0	0	1	3	1
Pituitary, focal hyperplasia of pars distalis	0	0	0	0	1

Termination at 104 weeks	Males (mg/kg/day)				
	0	0	20	60	200/100
Coagulating gland (N)	18	26	23	24	26
__distended	7	7	21	24	21
__interstitial lymphoid infiltration	1	1	18	18	16
__interstitial inflammation	1	0	1	0	2

__interstitial fibrosis	0	0	3	3	3
Seminal vesicle	18	26	23	24	26
__distension	12	16	21	24	21
__interstitial lymphoid infiltration	2	3	5	7	6
__interstitial fibrosis	0	0	2	5	0
Hepatocyte vacuolation, centrilobular	0	2	0	0	7
Pituitary, focal hyperplasia of pars distalis	0	0	0	0	2

All animals (N=50)	Males (mg/kg/day)				
	0	0	20	60	200/100
Coagulating gland , distension	18	20	44**	48**	23**
Coagulating gland					
__interstitial lymphoid infiltration, minimal	2	2	16	23	19
__interstitial lymphoid infiltration, slight	0	0	8	5	4
Coagulating gland					
__interstitial fibrosis, minimal	0	0	8	5	4
__interstitial fibrosis, slight	0	0	4	4	5
Seminal vesicle, distension	23	24	38*	44**	39*
Seminal vesicle,					
__interstitial lymphoid infiltration, minimal	2	3	7	8	6
__interstitial lymphoid infiltration, slight	0	0	0	0	3
Seminal vesicle,					
__interstitial fibrosis, minimal	0	0	3	6	1
__interstitial fibrosis, slight	0	0	0	2	0
Pituitary, focal hyperplasia of pars distalis	0	0	0	0	3

Neoplastic: No treatment related neoplasms were observed.

Toxicokinetics:

26 weeks	Males (mg/kg/day)			
	20	60	100	200
Cmax (ng/ml)	14.08	167.94	600	1794.22
Cmin (ng/ml)	4.10	24.72	500	108.40
AUC_{24} (nghr/ml)	229	1897	7500	22810

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The doses were adequate based on multiples of parent drug and metabolites for this non-genotoxic drug. All CAC and division suggestions were incorporated in final study. CAC concurred that the study was adequate.

Evaluation of tumor findings: No treatment related neoplasms were observed.

Study title: KMD-3213: Carcinogenicity Study by Dietary Administration to CD Rats for 104 Weeks

Key study findings: The thyroid follicular cell adenomas in male rats were statistically significant and were considered drug related. Although the incidence of follicular cell adenomas in female rats was increased, the incidences in dosed groups were not

statistically significant. There was increased incidence and severity, although minimal to slight, of thyroid follicular cell hypertrophy in the female rats, as well as in the male rats.

Study number: KSI 102/012989
Conducting laboratory and location: [redacted] b(4)

Date of study initiation: 20 May 1999
GLP compliance: yes
QA report: yes (x) no ( )
Drug: batches #JN301 (100.2 % pure), #JR261 (100.2 % pure), and #JZ011 (100.2 % pure)

Study Type: 2 yr bioassay
Species/strain: [redacted] CD® (SD) IGS BR rats b(4)
Number/sex/group; age at start of study: 60/group
Animal housing: by groups of 4 (satellite) or 5 (main study) according to sex
Formulation/vehicle: dietary
Drug stability/homogeneity: Confirmed weeks 1, 13, 26, 39, 52, 65, 78, 91, and 103

Methods:

Doses: 0, 0, 15, 50, and 150 mg/kg/day in males and 0, 0, 15, 80, 250 mg/kg/day in females
Basis of dose selection: AUC ratio
Route of administration: oral, dietary
Frequency of drug administration: dietary
Dual controls employed: yes

Results:

Mortality:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	50	150
Weeks 1-104*	28	24	31	25	31	37	34	26	34	29
Main group mortality (%)	47	40	52	42	52	62	57	43	57	48
Main group survival (%)	53	60	48	58	48	38	43	57	43	52

* Additionally, one control group 1 male, one control group 2 female, and one 15 mg/kg female were found dead during the period of the terminal kill

Clinical signs: Partially closed eyes were observed in animals from the mid and high dose groups.

Body weights:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	50	150
Weeks 1-104 gain (g/rat/week)	552	554	559	537	468**	331	357	338	318	248**
Weeks 1-104, % of comb.contr.	--	--	101	97	85	--	--	98	92	72

Food consumption:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	50	150
Weeks 1-104 (kg/rat/week)	20.7	20.0	20.5	20.3	19.5**	15.6	16.0	15.5	15.6	15.2*
Weeks 1-104, % of comb.cont.	--	--	101	100	96	--	--	98	98	96

Hematology: No treatment related effects were observed.
Clinical chemistry: Not measured.
Organ weights:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Term. body wt (g)	727.1	733.7	743.3	715.5	650.9	482.3	509.1	488.4	479.4	404.7
Liver										
__unadjusted	22.99	22.33	25.09	25.92	24.89	17.49	18.65	18.63	18.85	17.18
__adj. for body wt.	22.45	21.71	23.79*	25.36**	26.23**	17.09	17.33	18.02	18.55	19.45**
Thyroids + paras.										
__unadjusted	.050	.040	.044	.046	.056	.035	.039	.037	.039	.037
__adj. For body wt.	.043	.038	.040	.043	.054**	.034	.037	.036	.039	.040
Kidneys										
__unadjusted	4.94	4.83	5.42	4.90	4.47**	3.49	3.50	3.36	3.21	2.97
__adj. for body wt.	4.82	4.70	5.14	4.79	4.71	3.42	3.36	3.24	3.14*	3.13*
Lungs and bronchi										
__unadjusted	2.246	2.236	2.367	2.235	2.018**	1.832	1.697	1.729	1.681	1.586
__adj. For body wt.	2.193	2.174	2.244	2.186	2.127	1.795	1.665	1.708	1.664	1.615*
Salivary glands										
__unadjusted	.799	.806	.823	.832	.804	.560	.568	.555	.621	.602
__adj. For body wt.	.796	.800	.814	.832	.824	.555	.552	.548	.618**	.629**
Uterus + cervix	--	--	--	--	--	1.559	1.157	.852	.817**	.667**
Prostate	1.210	1.299	1.310	1.362	1.340	--	--	--	--	--

Gross pathology:

	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Animal thin	4/60	5/60	8/60	12/60	15/60	6/60	4/60	9/60	6/60	13/60
__<104 weeks	3/29	3/24	5/31	3/25	7/31	4/37	3/35	4/27	5/34	8/29
__104 weeks	1/31	2/36	3/29	9/35	8/29	2/23	1/25	5/33	1/26	5/31
Animal obese	4/60	3/60	3/60	1/60	2/60	3/60	5/60	5/60	2/60	0/60
__<104 weeks	2/29	0/24	0/31	1/25	1/31	1/37	0/35	0/27	2/34	0/29
__104 weeks	2/31	3/36	3/29	0/35	1/29	2/23	5/25	5/33	0/26	0/31

Histopathology:

Non-neoplastic:

< 104 weeks	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Liver (N)	29	24	31	25	31	37	35	27	34	29
__centrilobular vacuolation	9	7	8	12	11	1	3	4	2	2
__bile duct hyperplasia	13	14	6	9	16	12	9	4	9	8
Thyroids (N)	29	24	31	25	30	37	35	27	33	29
__follicular cell hypertrophy	1	1	1	2	5	1	0	0	3	7
__cystic follicular cell hyperplasia	0	1	1	1	3	2	0	0	0	1

104 weeks	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Liver (N)	31	36	29	35	29	23	25	33	26	31
__centrilobular vacuolation	13	11	6	15	21	5	4	4	1	2
__bile duct hyperplasia	14	16	12	21	19	5	7	9	14	19
Thyroids (N)	31	36	29	35	29	22	25	33	26	31
__follicular cell hypertrophy	1	3	9	9	6	0	0	1	0	7
__cystic follicular cell hyperplasia	0	0	3	2	2	0	0	0	0	1

Total of all animals	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Liver (N)	60	60	60	60	60	60	60	60	60	60
__centrilobular vacuolation	22	18	14	27	32	6	7	8	3	4
__bile duct hyperplasia	27	30	18	30	34	17	16	13	23	27
Thyroids (N)	60	60	60	60	59	59	60	60	59	60
__follicular cell hypertrophy	2	4	10*	11*	11**	1	0	1	3	14***
__cystic follicular cell hyperplasia	0	1	4	3	5	2	0	0	0	2

Neoplastic:

<104 weeks	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Thyroids (N)	29	24	31	25	30	37	35	27	33	29
__B-follicular cell adenoma	0	0	0	3	6	0	0	0	1	1
__M-follicular cell carcinoma	0	0	0	1	0	0	0	0	0	0

104 weeks	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Thyroids (N)	31	36	29	35	29	22	25	33	26	31
__B-follicular cell adenoma	1	0	1	1	6	1	0	2	1	3
__M-follicular cell carcinoma	1	1	0	0	2	0	0	0	0	0

Total of all animals	Males (mg/kg/day)					Females (mg/kg/day)				
	0	0	15	50	150	0	0	15	80	250
Thyroids (N)	60	60	60	60	59	59	60	60	59	60
__B-follicular cell adenoma	1	0	1	4	12	1	0	2	2	4
__M-follicular cell carcinoma	1	1	0	1	2	0	0	0	0	0

Male histor. data study code	A		B		C		D		E		F		G		H		I	
Kill (D=decedent, T=terminal)	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
Follicular cell adenoma	0	3	0	0	4	2	1	2	1	1	1	0	3	1	0	2	0	2
Follicular cell carcinoma	0	0	0	0	0	0	0	0	0	2	0	1	0	0	0	0	0	0
Number of thyroids examined	29	21	35	25	28	22	31	19	31	19	28	32	40	25	39	26	29	36

Female histor. data study code	A		B		C		D		E		F		G		H		I	
Kill (D=decedent, T=terminal)	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T	D	T
Follicular cell adenoma	1	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0
Follicular cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Number of thyroids examined	31	19	44	16	34	16	36	13	33	17	36	24	43	22	40	24	44	21

Toxicokinetics:

26 weeks	Males (mg/kg/day)			Females (mg/kg/day)		
	15	50/80	150/250	15	50/80	150/250
Cmax (ng/ml)	34.49	115.66	675.78	23.85	152.49	471.91
Cmin (ng/ml)	--	51.83	216:53	--	42.31	182.84
AUC24 (nghr/ml)	422	2011	9509	261	2235	8282

Summary of individual study findings:

Adequacy of the carcinogenicity study and appropriateness of the test model: The study was adequate doses based on multiples of parent drug and metabolites for this non-genotoxic drug. All CAC and division suggestions were incorporated in final study. CAC concurred that the study was adequate.

Evaluation of tumor findings: The thyroid follicular cell adenomas in male rats were statistically significant and were considered drug related. Although the incidence of follicular cell adenomas in female rats was increased, the incidences in dosed groups were not statistically significant. There was also increased incidence and severity, although minimal to slight, of thyroid follicular cell hypertrophy in the female rats, as well as in the male rats.

Carcinogenicity summary:

The rat and mouse studies were adequate.

The thyroid follicular cell adenomas in male rats were statistically significant and considered drug related. Although the incidence of follicular cell adenomas in female rats was increased, the incidences in dosed groups were not statistically significant. There was also increased incidence and severity, although minimal to slight, of thyroid follicular cell hypertrophy in the female rats, as well as in the male rats

Mammary gland adenoacanthomas, mammary gland adenocarcinomas, and mammary gland adenomas or carcinomas were statistically significant and were considered drug related in female mice.

There were no drug related neoplasms in male mice.

Carcinogenicity conclusions:

CAC concurred that the thyroid follicular cell adenomas in male rats were drug related. Although the incidence of follicular cell adenomas in female rats was increased, the incidences in dosed groups were not statistically significant and thus not clearly related to the drug. However, the committee noted that the increased incidence and severity, although minimal to slight, of thyroid follicular cell hypertrophy in the female rats, as well as in the male rats, suggests that the thyroid of females is also a potential organ for toxicity of the drug. The Committee concurred that the mammary gland adenoacanthomas were drug related. It also concurred that the mammary gland adenocarcinomas, and adenomas or carcinomas, were drug related and that there were no drug-related neoplasms in male mice.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Study of KMD-3213 by oral administration to rats prior to and in the early stages of pregnancy

Key study findings: In a combined male/female rat fertility study, at 60 mg/kg/day and above, prolongation or disappearance of the estrous cycle was observed in females. Decreased copulation index was observed at 200 mg/kg/day and above and decreased fertility index was observed at 20 mg/kg/day and above (all treated doses).

Study no.: 10006
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano 399-83, Japan
Date of study initiation: 15 February 1995
GLP compliance: yes, Japanese guidelines
QA reports: yes (x) no ( )
Drug, lot #, and % purity: KMD-3213, Lot No.: GD231, 99.6% pure

Methods b(4)

Doses: 0, 20, 60, 200, and 600 mg/kg/day
Species/strain: rat — :CD(SD) (Sprague-Dawley, SPF)
Number/sex/group: 25 (males, age 6 weeks, 216.4 – 256.2 g and females, age 11 weeks, 236.6 – 283.8 g)
Route, formulation, volume, and infusion rate: oral (gavage) in 0.5% methylcellulose
Study design:
Period of administration: Females were treated for 15 days before mating and through the mating period up to gestation Day 7, and males were treated for 64 days before mating and through the mating period up to the day before necropsy. C-sections were performed on Day 20.

Results

Mortality/Clinical signs: No treatment-related deaths occurred. Clinical signs, observed in all treatment groups, included ptosis and lacrimation, which were considered to be due to the pharmacological action of KMD-3213.

Body weight: Body weights were decreased in male rats treated with 600 mg/kg KMD-3213 compared with vehicle control animals. Body weight gains were depressed in pregnant female rats treated at 600 mg/kg. There were decreases in food consumption in male and female rats, corresponding to the decreased body weights and depressed body weight gains.

Fertility parameters: Decreased copulation index was observed at 200 mg/kg/day and above, and decreased fertility index was observed at 20 mg/kg/day and above.

Dose (mg/kg/day)	0 (Control)	20	60	200	600
Male					
Toxicokinetics					
AUC_{0-t} [a] (ug·hr/mL) Day 1	Not done	866.03	1578.07	3493.72	3642.84
Month 1	Not done	701.33	2567.67	11150.89	24241.20
Number of animals evaluated	25	25	25	25	25
Number of animals died or sacrificed moribund	0	0	0	0	0
Clinical signs:					
Eyelid ptosis	-	+	+	+	+
Lacrimation	-	+	+	+	+
Salivation	-	-	-	+	+
Necropsy	-	-	-	-	-
Body weight [b] (g) [d]	568.0 ± 66.3	592.5 ± 56.3	573.1 ± 45.0	577.6 ± 64.4	526.3 ± 54.4*
Food consumption [c] (g/day) [d]	29.3 ± 3.8	29.8 ± 3.6	31.3 ± 2.3	29.6 ± 3.5	25.3 ± 3.4**
Food consumption [b] (g/day) [d]	30.8 ± 4.6	31.6 ± 4.2	30.5 ± 3.6	32.1 ± 4.2	29.9 ± 3.9
Organ weight [d]	-	-	-	-	-
Relative testes	6.06±0.87	5.70±1.15	5.99±0.65	6.15±0.78	6.68±0.86*
Number of males that mated	25	25	23	14	16
Number of fertile males	22	12	6	6	6
Copulation index (%) [e]	100.0	100.0	92.0	56.0**	64.0**
Fertility index (%) [e]	88.0	48.0**	26.1**	42.9**	37.5**

Mean ± S.D. G=Gestation day.

-: No noticeable finding, +: Finding noted.

a) Study No. 10026, b) Week 9, c) Day 11, d) Dunnett's multiple comparison test (vs control), e) $\chi 2$ test (vs control): *; $p<0.05$ **; $p<0.01$.

At 60 mg/kg/day and above, prolongation or disappearance of the estrous cycle was observed. Decreased fertility index was observed at 20 mg/kg/day and above

Dose (mg/kg/day)	0 (Control)	20	60	200	600
Female					
Toxicokinetics					
AUC_{0-t} [a] (ug·hr/mL) Day 1	Not done	1593.73	1908.72	3969.32	4957.85
Month 1	Not done	531.68	1857.70	4105.87	7518.73
Number of animals evaluated	25	25	25	25	25
Number of animals died or sacrificed moribund	0	0	0	1[e]	0
Clinical signs:					
Eyelid ptosis	-	+	+	+	+
Lacrimation	-	+	+	+	+
Necropsy	-	-	-	-	-
Body weight before mating (g) [b],[d]	277.6 ± 15.6	292.8 ± 18.3*	307.2 ± 20.0**	314.9 ± 25.7**	312.9 ± 19.1**
Body weight gain during gestation (g) [b],[d]	46.3 ± 8.3	49.4 ± 12.4	39.6 ± 12.6	39.0 ± 7.0	18.9 ± 3.8**
Food consumption before mating (g) [b],[d]	18.3 ± 2.8	21.7 ± 3.2**	22.9 ± 3.0**	21.8 ± 4.0**	21.2 ± 3.4**
Food consumption during gestation (g) [b],[d]	29.8 ± 3.1	30.4 ± 3.4	29.0 ± 3.1	29.2 ± 3.6	23.0 ± 3.2**
Estrous cycle [b]	-	-	+[f]	+[f]	+[f]
Number of females that mated	24	23	22	13	16
Number of fertile females	23	11	7	6	6
Copulation index (%) [c]	96.0	92.0	88.0	54.2**	64.0*
Fertility index (%) [c]	95.8	47.8**	31.8**	46.2**	37.5**
Number of dams evaluated	21	8	7	5	6
Mean number of corpora lutea [b]	18.1 ± 1.68	17.8 ± 2.19	17.7 ± 1.60	19.0 ± 2.65	16.7 ± 4.68
Mean number of implantation [b]	17.0 ± 3.54	14.0 ± 5.21	16.0 ± 3.32	18.2 ± 3.27	12.8 ± 6.40

Mean ± S.D., -: No noticeable finding, +: Finding noted.

a) Study No. 10026, b) Dunnett's multiple comparison test (vs control), c) $\chi 2$ test (vs control): *; $p<0.05$ **; $p<0.01$,

d) At the final point before mating or at the end of administration during gestation period (Gestation Day 8),

e) Sacrificed due to gavage error. , f) Prolonged or disappeared estrous cycle.

Dose (mg/kg/day)		0 (Control)	20	60	200	600
Litters						
Mean implantation index (%) c)		92.9 ± 16.01	78.9 ± 27.96	89.9 ± 13.88	95.4 ± 5.15	71.3 ± 32.60**
Mean number of live fetuses a)		15.3 ± 3.57	12.5 ± 4.93	15.7 ± 2.93	16.8 ± 2.28	12.3 ± 6.74
Mean postimplantation loss (%) c)		9.6	10.7	1.8**	7.7	3.9*
Sex ratio of fetus (M/F) b)		0.85	0.85	1.08	0.95	0.68
Mean fetal body weight (g) a)	M:	3.54 ± 0.24	3.51 ± 0.24	3.55 ± 0.34	3.36 ± 0.18	2.97 ± 1.48
	F:	3.35 ± 0.21	3.42 ± 0.18	3.39 ± 0.25	3.21 ± 0.22	2.86 ± 1.45
Fetal abnormality:						
External abnormality						
Number of fetus with mandibular cleft c)		0	0	1 (0.9%)	0	0

Mean ± S.D.

a) Dunnett's multiple comparison test (vs control), b) χ^2 test (vs control),

b) χ^2 test (vs control). * $p<0.05$, ** $p<0.01$

c) Wilcoxon's rank sum test (vs control): *; $p<0.05$ **; $p<0.01$.

Study title: Study by oral administration of KMD-3213 for fertility and early embryonic development to implantation in rats treated orally; Assessment by administration to males

Key study findings: Sperm viability and count were significantly lower after administration of 600 mg/kg/day (about 65 times the exposure of the maximum recommended human dose via AUC) for one month. Histopathological examination of infertile males revealed changes in the testes and epididymides at 200 mg/kg/day (about 30 times). Reduction in male fertility did not reach statistical significance in this study, although it was observed in two other studies at 20 mg/kg/day.

Study no.: 10059
Conducting laboratory and location: Toxicology Laboratory, RAD, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano 399-83, Japan
Date of study initiation: 8 April 1996
GLP compliance: Japanese
QA reports: yes (x) no ( )
Drug, lot #, and % purity: KMD-3213, Lot No.: GT081, 100.1% pure

Methods

Doses: 0, 20, 60, 200, and 600 mg/kg/day
Species/strain: rat, Sprague-Dawley
Number/sex/group: 20
Route, formulation, volume, and infusion rate: oral (gavage), 5 ml/kg in 0.5% methylcellulose
Satellite groups used for toxicokinetics:
Study design:
Duration of administration: 29 pre-mating days and during the mating period up to the day before necropsy (in males).

Results

Mortality: One male in the 600 mg/kg/day group died on Day 3 of treatment.

Clinical signs: Ptosis and lacrimation were observed in all treated groups and were considered to be ascribable to the pharmacology of KMD-3213.

Body weight: Body weights of male rats were lower in the 600 mg/kg/day group than in the control group, although the difference was not significant.

Food consumption: Food consumption was significantly or slightly lower in the 600 mg/kg/day group than in the control group.

Toxicokinetics:

Dose (mg/kg/day)	0 (Control)	20	60	200	600
Male					
Toxicokinetics					
AUC_{0-4} [a] (μg·hr/mL) Day 1	Not done	866.03	1578.07	3493.72	3642.84
Month 1	Not done	701.33	2567.67	11150.89	24241.20
Number of animals evaluated	20	20	20	20	20
Number of animals dead and slaughtered moribund	–	–	–	–	2 [b]

Mean ± S.D. G=Gestation Day.

-: No noticeable finding.

a) Study No. 10026, b) One animal died due to gavage error on Day 34, and another died on Day 3.

Necropsy: At terminal necropsy, yellowish change of the liver and accentuated lobular pattern were observed macroscopically in many males treated at 600 mg/kg/day and granular nodule in the right caudal epididymis was noted in 1 male in the 600 mg/kg/day group.

Fertility parameters: Sperm viability and count were significantly lower after administration of 600 mg/kg/day. Aspermatogenesis was observed at 200 mg/kg/day. Reduction in male fertility did not reach statistical significance.

Dose (mg/kg/day)	0 (Control)	20	60	200	600
Male					
Clinical signs					
Eyelid ptosis	–	+	+	+	+
Lacrimation	–	+	+	+	+
Necropsy					
Liver:					
Yellow coloring or clearer lobular pattern	0	0	0	0	13
Body weight [a] (g) [b]	502.6 ± 30.0	501.9 ± 23.5	512.7 ± 30.4	510.4 ± 28.9	483.3 ± 35.0
Food consumption [c] (g/day) [b]	27.9 ± 2.9	28.8 ± 2.8	29.4 ± 3.2	28.4 ± 2.9	25.1 ± 3.3*
Food consumption [a] (g/day) [b]	28.6 ± 2.8	29.8 ± 2.6	30.6 ± 2.8	31.9 ± 3.1**	28.5 ± 3.9
Sperm test					
Sperm survival rate (%) [b]	91.1 ± 2.2	91.7 ± 2.5	90.3 ± 3.8	86.0 ± 19.2	58.8 ± 35.9**
Number of sperm (×10⁶/g of epididymis) [b]	673.1 ± 80.0	648.2 ± 81.1	640.6 ± 111.8	619.3 ± 136.0	466.6 ± 163.6**
Organ weight [b]	–	–	–	–	–
Histopathology (infertile animals)					
Aspermatogenesis	–	–	–	+	+
Number of males that mated	20	20	20	20	19
Number of fertile males	18	18	12	17	12
Copulation index (%) [d]	100.0	100.0	100.0	100.0	100.0
Fertility index (%) [d]	90.0	90.0	60.0	85.0	63.2

Mean ± S.D. G=Gestation Day.

-: No noticeable finding, +: Finding noted.

a) At the final point before mating, b) Dunnett's multiple comparison test (vs control): *: $p<0.05$ **: $p<0.01$, c) Day 12,

d) χ^2 test (vs control).

Histopathological changes were observed in 1/8 rats in the 60 mg/kg/day group, 1/3 rats in the 200 mg/kg/day group, and 7/7 rats in the 600 mg/kg/day group. Mild focal sloughing of spermatids was observed in the seminiferous tubules for one rat each in the 60 and 200 mg/kg/day groups and for two rats in the 600 mg/kg/day group. Retention of cell debris in the epididymal ducts was observed at 200 (1 rat) and 600 mg/kg/day (2 rats). In 5 rats of the 600 mg/kg/day group, the testes and epididymis showed aspermatogenesis (atrophy and/or degeneration of seminiferous tubules, focal sloughing of spermatids, epididymal luminal retention of cell debris or spermatic granuloma). One rat in the 20 mg/kg/day group showed atrophy of the right testis and epididymis, aspermatogenesis, and diffuse hyperplasia of Leydig cells in the testicular stroma.

	Males rats (mg/kg/day)				
	0	20	60	200	600
Mild focal sloughing of spermatids in seminiferous tubules			1	1	2
Retention of cell debris in epididymal ducts				1	2
Aspermatogenesis (atrophy and/or degeneration of seminiferous tubules, focal sloughing of spermatids, epididymal luminal retention of cell debris or spermatic granuloma)					5
Macroscopic atrophy of the right testis and epididymis, with aspermagenesis and diffuse hyperplasia		1			

Mated/untreated females showed a decrease in implantation index at 600 mg/kg/day.

Dose (mg/kg/day)	0 (Control)	20	60	200	600
Females (untreated)					
Number of animals evaluated	18	18	11	17	12
Clinical signs	-	-	-	-	-
Necropsy	-	-	-	-	-
Mean number of corpora lutea [a]	17.9 ± 2.27	16.8 ± 2.49	16.8 ± 2.18	16.9 ± 2.28	16.1 ± 2.35
Mean number of implantations [a]	16.5 ± 3.73	12.6 ± 5.41	12.9 ± 5.84	13.3 ± 6.01	11.3 ± 6.14
Implantation index (%) [b]	91.8 ± 17.40	72.8 ± 28.62*	75.8 ± 33.37	75.8 ± 30.74	68.7 ± 33.58*
Mean number of surviving embryos [a]	15.1 ± 3.67	12.1 ± 5.53	12.4 ± 5.57	12.1 ± 5.98	10.7 ± 5.79
Mean postimplantation loss (%) [b]	8.8	4.0*	4.2*	8.8	5.9

Mean ± S.D.

-: No noticeable finding, +: Finding noted.

a) Dunnett's multiple comparison test (vs control).

b) Wilcoxon's rank sum test (vs control): *: $p<0.05$ **: $p<0.01$.

Study title: Study by oral administration of KMD-3213 for fertility and early embryonic development to implantation in rats; Additional study for the assessment by administration to males

Key study finding: Treatment of male rats with silodosin for 15 days resulted in decreased fertility at the high dose of 20 mg/kg/day (about twice the exposure of the maximum recommended human dose via AUC) which was reversible following a two week recovery period. No effect was observed at 6 mg/kg/day.

Study no.: 10112, KMD-TX1998-403E01
Conducting laboratory and location: Toxicology Laboratories, R&D Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagano-pref., 399-83 Japan
Date of study initiation: 7 October 1997
GLP compliance: yes

QA reports: yes (x) no ( )
Drug, lot #, and % purity: KMD-3213, Lot No. JH312, 100.1 % pure

Methods

Doses: 0, 2, 6, and 20 mg/kg/day
Species/strain: rat, Sprague-Dawley
Number/sex/group: 20
Route, formulation, volume, and infusion rate: oral (gavage) in 0.5% methylcellulose

Study design:

Period of administration: In males, KMD-3213 was administered for 4 week premating and during the mating period -1(for 16 days). Following a 2-week recovery period, treated males were mated again for 20 days.

Results

Mortality:

Clinical signs: Ptosis and lacrimation were observed in all the KMD-3213-treated groups during the treatment period and these symptoms were considered to be due to the pharmacological effects of KMD-3213.

Body weight: No treatment related effects were observed.

Toxicokinetics:

Dose (mg/kg/day)	0 (Control)	2	6	20
Male				
Toxicokinetics				
AUC_{0-t} [a] (ng·hr/mL) Day 1	Not done	Not done	Not done	866.03
Month 1	Not done	Not done	Not done	701.33
Number of animals evaluated	20	20	20	20
Number of animals dead or sacrificed moribund	0	0	0	0

Mean ± S.D. G=Gestation Day.
a) Study No. 10026.

Necropsy: No treatment related effects were observed.

Fertility parameters : A decrease in fertility was observed in the 20 mg/kg/day group after mating I, but no decrease in fertility was observed after mating II, two week after discontinuation of dosing.

Dose (mg/kg/day)	0 (Control)	2	6	20
Male				
Clinical signs				
Eyelid prosis	–	+	+	+
Lacrimation	–	–	+	+
Necropsy	–	–	–	–
Body weight [a] (g) [b]	430.1 ± 31.2	424.2 ± 27.2	430.9 ± 31.7	420.2 ± 29.7
Food consumption [a] (g/day) [b]	28.4 ± 2.8	27.2 ± 2.5	28.6 ± 3.0	28.5 ± 2.9
Mating I				
Number of Males that mated	20	20	20	20
Number of fertile males	20	20	16	15
Copulation index (%) [c]	100.0	100.0	100.0	100.0
Fertility index (%) [c]	100.0	100.0	80.0	75.0*
Mating II				
Number of females that mated	20	20	20	19
Number of fertile females	19	19	17	18
Copulation index (%) [c]	100.0	100.0	100.0	95.0
Fertility index (%) [c]	95.0	95.0	85.0	94.7

Mean ± S.D. G=Gestation Day.

No noticeable finding. +: Finding noted.

a) At the final point before mating, b) Dunnett's multiple comparison test (vs control), c) χ^2 test (vs control): *; $p<0.05$.

Dose (mg/kg/day)	0 (Control)	2	6	20
Female (Untreated)				
Mating I				
Number of animals evaluated	20	20	20	20
Clinical signs	–	–	–	–
Necropsy	–	–	–	–
Mean number of corpora lutea [a]	16.9 ± 1.71	17.5 ± 2.01	16.9 ± 1.57	16.5 ± 1.88
Mean number of implantations [a]	15.4 ± 3.70	16.8 ± 1.77	15.8 ± 3.19	13.0 ± 4.78
Implantation index (%) [b]	90.8 ± 20.60	96.2 ± 4.28	92.9 ± 17.01	77.8 ± 25.32*
Mean number of surviving embryo [a]	14.8 ± 3.61	15.8 ± 1.77	14.3 ± 3.24	12.1 ± 4.56
Mean postimplantation loss (%) [b]	3.7 ± 4.1	6.1 ± 5.8	9.0 ± 8.2	6.9 ± 6.6
Mating II				
Number of animals evaluated	20	20	20	20
Clinical signs	–	–	–	–
Necropsy	–	–	–	–
Mean number of corpora lutea [a]	17.3 ± 2.00	16.2 ± 1.65	16.5 ± 2.18	16.9 ± 1.86
Mean number of implantations [a]	16.1 ± 2.60	15.3 ± 1.52	15.2 ± 4.02	16.1 ± 2.82
Implantation index (%) [b]	93.3 ± 12.24	94.4 ± 7.09	91.4 ± 20.42	94.3 ± 11.25
Mean number of surviving embryo [a]	14.6 ± 2.77	14.2 ± 1.65	14.2 ± 4.02	15.0 ± 2.57
Mean postimplantation loss (%) [b]	9.3 ± 7.2	6.7 ± 7.9	6.3 ± 8.7	6.2 ± 6.9

Mean ± S.D. G=Gestation Day.

-: No noticeable finding.

a) Dunnett's multiple comparison test (vs control). b) Wilcoxon's rank sum test (vs control): *; $p<0.05$.

Study title: Study by oral administration of KMD-3213 for fertility and early embryonic development to implantation in rats; assessment by administration to females

Key study findings: In a fertility study in female rats, the high dose of 20 mg/kg/day (about 1 to 4 times the exposure of the maximum recommended human dose via AUC) resulted in estrus cycle changes, but no effect on fertility. No effect on the estrus cycle was observed at 6 mg/kg/day.

Study no.: 10072
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd. 2320-1, Maki, Hotaka, Minamiazumi, Nagano 399-83, Japan
Date of study initiation: 28 June 1996
GLP compliance: Japanese MHW
QA reports: yes (x) no ( )
Drug, lot #, and % purity: KMD-3213, Lot No. GD231, 99.6% pure

Methods

Doses: 0, 0.6, 2, 6, and 20 mg/kg/day
Species/strain: rat, Sprague-Dawley, SPF
Number/sex/group: 20
Route, formulation, volume, and infusion rate: oral (gavage) in 5 ml/kg 0.5% methylcellulose
Study design: Dosing from 15 days pre-mating until gestation Day 7
Duration of administration: During the 15 pre-mating days through the mating period up to gestation Day 7 (in females).

Results

Mortality: No deaths occurred during the study.

Clinical signs: Lacrimation was observed in the 2, 6, and 20 mg/kg/day groups and ptosis was observed in the 6 and 20 mg/kg/day groups during treatment.

Body weight/Food consumption: No treatment related effects were observed.

Toxicokinetics:

Dose (mg/kg/day)	0 (Control)	0.6	2	6	20
Female					
Toxicokinetics					
AUC_{0-t} [a] (μg·hr/mL) Day 1	Not done	Not done	Not done	Not done	1593.73
Month 1	Not done	Not done	Not done	Not done	531.68
Number of animals evaluated	20	20	20	20	20
Number of animals indeed or sacrificed moribund	0	0	0	0	0

Mean ± S.D. G=Gestation Day.
a) Study No. 10026.

Fertility parameters: At 20 mg/kg/day, no effects on fertility were observed.

Dose (mg/kg/day)	0 (Control)	0.6	2	6	20
Female					
Clinical signs					
Eyelid ptosis	–	–	–	+	+
Lacrimation	–	–	+	+	+
Necropsy	–	–	–	–	–
Body weight before mating (g) a),b)	288.7 ± 15.2	293.4 ± 13.1	292.1 ± 13.6	291.3 ± 12.4	298.1 ± 18.3
Body weight gain during gestation (g) a),b)	49.5 ± 10.8	49.9 ± 9.4	46.3 ± 10.2	45.3 ± 7.6	46.0 ± 9.4
Food consumption before mating (g) a),b)	20.2 ± 3.3	20.7 ± 2.2	20.1 ± 2.5	20.9 ± 2.8	22.2 ± 3.2
Food consumption during gestation (g) a),b)	29.3 ± 3.6	29.2 ± 3.1	28.5 ± 3.1	28.5 ± 2.4	29.3 ± 4.0
Estrous cycle b)	–	–	–	–	–
Mean number of days prior to mating b)	2.4 ± 1.3	3.6 ± 2.6	2.8 ± 1.1	2.6 ± 1.0	3.6 ± 3.2
Number of females that mated	20	20	20	20	20
Number of fertile females	19	20	20	20	20
Copulation index (%) c)	100.0	100.0	100.0	100.0	100.0
Fertility index (%) c)	95.0	100.0	100.0	100.0	100.0
Mean number of corpora lutea b)	18.3 ± 2.31	17.8 ± 2.05	18.1 ± 2.46	17.8 ± 2.09	18.1 ± 1.93
Mean number of implantations b)	16.7 ± 2.24	16.9 ± 2.80	17.0 ± 3.07	16.7 ± 2.18	16.2 ± 3.42
Implantation index (%) d)	91.6 ± 8.80	94.8 ± 11.33	93.4 ± 11.22	93.6 ± 6.36	89.7 ± 17.54
Mean number of surviving embryo b)	15.5 ± 2.44	15.6 ± 3.17	16.1 ± 3.11	16.0 ± 1.84	14.9 ± 3.99
Mean postimplantation loss(%) d)	7.3 ± 7.2	7.2 ± 12.0	5.4 ± 5.4	3.6 ± 4.7	9.9 ± 15.0

Mean ± S.D.

-: No noticeable finding. +: Finding noted.

a) At the final point before mating period or at the end of administration during gestation period (Gestation Day 8).

b) Dunnett's multiple comparison test (vs control), c) χ^2 test (vs control), d) Wilcoxon's rank sum test (vs. control).

Mild estrus cycle changes were observed at 20 mg/kg/day, but not at 6 mg/kg/day.

Dose (mg/kg)	Estrous cycle (Mean ± S.D.)			
	Estrus	Metestrus-1	Metestrus-2	Diestrus
Control	0.00	3.90 ± 0.72	3.35 ± 0.88	7.75 ± 0.91
0.6	0.10 ± 0.45	3.80 ± 0.70	3.65 ± 0.59	7.45 ± 0.69
2	0.00	3.60 ± 0.75	3.40 ± 0.99	8.00 ± 1.65
6	0.00	3.85 ± 0.37	3.60 ± 0.50	7.55 ± 0.69
20	0.00	3.25 ± 1.16 *	3.15 ± 0.88	8.60 ± 1.73

*: Significant difference from control at $p<0.05$

APPEARS THIS WAY
ON ORIGINAL

Embryofetal development

Study title: Preliminary study by oral administration of KMD-3213 for effects on embryo-fetal development in rats.

Key study findings: A high dose of 700 mg/kg was chosen for the main study since no toxicity was observed at 600 mg/kg (ptosis and lacrimation was observed at all doses) and since 800 mg/kg was shown to be lethal in a previous study.

Study no.: 50033
Submission #046, Volume #2, and page #848
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagao 399-8305, Japan
Date of study initiation: 22 January 1996
GLP compliance:
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: KMD-3213, lot # GT081, 100.1 % pure

Methods

Doses: 0, 200, 400, and 600 mg/kg
Species/strain: Rat, Sprague-Dawley, SPF
Number/sex/group: 10 females/group
Route, formulation, volume, and infusion rate: oral, in 0.5% methylcellulose
Study design: administration daily, days 7-17 of gestation
Parameters and endpoints evaluated: body weights, food consumption, corpora lutea, implantations, live fetuses, embryo-fetal mortality, sex ratio, fetal body weights, external anomalies.

Study title: Preliminary study by oral administration of KMD-3213 for effects on embryo-fetal development in rabbits.

Key study findings: 200 mg/kg was chosen as the high dose group in the main study due to decreases in body weight and food consumption at this dose and lethality at 400 mg/kg.

Study no.: 50034
Submission #046, Volume #2, and page #860
Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd., 2320-1, Maki, Hotaka, Minamiazumi, Nagao 399-8305, Japan
Date of study initiation: 13 December 1995
GLP compliance:
QA reports: yes ( ) no (x)
Drug, lot #, and % purity: KMD-3213, lot # GX201,

Methods

Doses: 0, 50, 100, 200, 400, and 600 mg/kg/day

Species/strain: rabbit, NZW, SPF
Number/sex/group: 5
Route, formulation, volume, and infusion rate: oral , in 0.5% methylcellulose
Study design: daily from days 6-18 of gestation
Parameters and endpoints evaluated: corpora lutea, implantations, live fetuses, sex ratio, fetal body weight, external anomalies

Results

Mortality (dams): 3/4 dams in the 400 mg/kg and 5/5 dams in the 600 mg/kg group died. Multifocal red spots or ulceration of the stomach were observed in some dams that died.

Clinical signs (dams):

	Dams (mg/kg/day)					
	0	50	100	200	400	600
Abortion	1			1	2	
Loose stool				1		1
Emaciation				1	1	1
Hematuria					1	
Ptosis					3	2
Lacrimation					1	
Vaginal bleeding					1	
Decrease of spont. act.						1
Soiling of inguinal reg.						1
Death					3	5

Body weight (dams): decreased at 200 mg/kg and above

Food consumption (dams): decreased at 200 mg/kg and above

Offspring (malformations, variations, etc.): One fetus in the 200 mg/kg group exhibited gastroschisis, anury, anal atresia and club foot.

Study title: Study by oral administration of KMD-3213 for effects on embryo/ fetal development in rats

Key study findings: A NOAEL for embryo-fetal development was judged to be greater than 700 mg/kg/day when KMD-3213 was administered orally to pregnant rats during the fetal organogenesis period.

Study no.: 10058
Conducting laboratory and location: Toxicology Research Laboratory, R&D Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 7 March 1996
GLP compliance: yes, Japanese

QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot no. GT081, 100.1% pure

Methods

Doses: 0, 30, 80, 240, and 700 mg/kg/day b(4)
Species/strain: rat — CD(SD) (Sprague-Dawley, SPF)
Number/sex/group: 20
Route, formulation, volume, and infusion rate: oral, in 5 ml/kg 0.5% methylcellulose solution
Study design: treatment from Day 7 to Day 17 of gestation

Results

Mortality (dams): No deaths occurred during the study period.

Clinical signs (dams): Clinical signs observed during the treatment period, at all dose levels, included ptosis and lacrimation.

Body weight (dams): No treatment related effects were observed.

Food consumption (dams): No treatment related effects were observed.

Terminal and necroscopic evaluations: C-section data: No effect of treatment was observed on the number of corpora lutea, the number of implantations, the number of live fetuses, the number of dead fetuses, sex ratio or fetal body weights.

Offspring (malformations, variations, etc.): External abnormalities observed included gastroschisis, oligodactyly of fore limbs and kinky tail in one fetus in the control group, omphalocele in one fetus in the 80 mg/kg group, and microsomia and vestigial tail in one fetus in the 700 mg/kg group. Visceral examination revealed a left umbilical artery in 4 and 1 fetuses in the control and 700 mg/kg groups, respectively. No similar abnormality occurred in the 30, 80 and 240 mg/kg groups. Thymic remnant in the neck was detected in 5 - 18 fetuses in every group including the control group, and vascular ring and abnormal origin of the right subclavian artery were found in 1 fetus/litter in the 30 mg/kg group. The incidences of these abnormalities showed no significant differences between the treated and control groups. In addition, dilatation of the renal pelvis was observed in 1 fetus in each of the control and 80 mg/kg groups and 2 fetuses in the 240 mg/kg group, and dilatation of ureter was seen in 1 fetus in each of the 30 and 240 mg/kg groups. The incidences of these variations demonstrated no significant differences between the treated and control groups. Skeletal examination disclosed no abnormalities in any fetus in any group. All skeletal variations observed represented those which typically occur and the incidence of variation showed no significant differences between the KMD-3213-treated groups and the control group. The indices of ossification progression (the numbers of metacarpal bones, metatarsal bones and sacral/caudal vertebrae and the incidence of retarded ossification) demonstrated no significant differences between the KMD-3213

treated groups and the control group.

Group and Dose	Control 0mg/kg	KMD-3213 30mg/kg	KMD-3213 80mg/kg	KMD-3213 240mg/kg	KMD-3213 700mg/kg
(External anomalies)					
No. of fetuses observed	328	299	326	312	299
No. of fetuses with anomalies(%)	1(0.3)	0	1(0.3)	0	1(0.3)
Types					
Gastroschisis(%)	1(0.3)	0	0	0	0
Omphalocele(%)	0	0	1(0.3)	0	0
Microsomia(%)	0	0	0	0	1(0.3)
Kinky tail(%)	1(0.3)	0	0	0	0
Vestigial tail(%)	0	0	0	0	1(0.3)
Oligodactyly of fore limb(%)	1(0.3)	0	0	0	0
(Visceral anomalies)					
No. of fetuses observed	169	151	167	161	152
No. of fetuses with anomalies(%)	17(10.1)	7(4.6)	18(10.8)	10(6.2)	8(5.3)
Types					
Thymic remnant in the neck(%)	14(8.3)	5(3.3)	18(10.8)	10(6.2)	7(4.6)
Abnormal origin of right subclavian artery(%)	0	1(0.7)	0	0	0
Vascular rings(%)	0	1(0.7)	0	0	0
Left umbilical artery(%)	4(2.4)	0 *	0 *	0 *	1(0.7)
(Skeletal anomalies)					
No. of fetuses observed	158	148	158	151	146
No. of fetuses with anomalies	0	0	0	0	0

* : Significant difference from control at p<0.05

Daily Dose (mg/kg)	0 (Control)	30	80	240	700
Dams:					
Number of pregnant animals	20	20	20	19	19
Clinical signs					
Eyelid ptosis	-	+	+	+	+
Lacrimation	-	+	+	+	+
Necropsy	-	-	-	-	-
Body weight gain (g) a),b)	136.0 ± 16.4	138.5 ± 18.3	141.7 ± 21.0	142.5 ± 21.0	128.8 ± 21.1
Food consumption (g/day) a),b)	29.6 ± 4.3	31.0 ± 4.3	31.8 ± 4.1	31.4 ± 3.9	30.1 ± 2.8
Mean number of corpora lutea b)	17.7 ± 1.17	17.4 ± 2.35	18.2 ± 1.99	18.5 ± 1.87	17.4 ± 2.01
Mean number of implantations b)	17.2 ± 1.20	15.8 ± 4.29	17.5 ± 2.14	17.5 ± 2.17	16.3 ± 3.74
Litters:					
Number of litters evaluated	20	20	20	19	19
Mean number of surviving fetuses b)	16.4 ± 1.67	15.0 ± 4.14	16.3 ± 2.41	16.4 ± 2.19	15.7 ± 3.57
Mean implantation index (%) c)	97.2 ± 3.82	88.8 ± 18.13	96.1 ± 4.90	94.6 ± 7.43	93.4 ± 18.19
Mean postimplantation loss(%) c)	4.7	5.1	6.9	6.0	3.5
Mean fetal body weight (g) b)					
Male	3.55 ± 0.22	3.52 ± 0.23	3.63 ± 0.22	3.62 ± 0.43	3.68 ± 0.25
Female	3.36 ± 0.18	3.34 ± 0.21	3.42 ± 0.17	3.40 ± 0.42	3.48 ± 0.24
Fetal sex ratios (M/F) d)	1.05	0.98	0.82	0.95	0.80
Fetal abnormalities:					
Number of fetus with external abnormality c)	1 (0.3%)	0	1 (0.3%)	0	1 (0.3%)
Number of fetus with visceral abnormality c)	17 (10.1%)	7 (4.6%)	18 (10.8%)	10 (6.2%)	8 (5.3%)
Number of fetus with skeletal abnormality c)	0	0	0	0	0

Mean ± S.D.

-: No noticeable finding, +: Finding noted.

a) At the end of treatment (Gestation Day 18), b) Dunnett's multiple comparison test (vs control).

c) Wilcoxon's rank sum test (vs control), d) $\chi 2$ test (vs control).

Study title: Study by oral administration of KMD-3213 for effects on embryo-fetal development in rats (2)

Key study findings: A NOAEL for embryo-fetal development was judged to be 1000 mg/kg/day when KMD-3213 was administered orally to pregnant rats during the fetal organogenesis period. This dose was roughly estimated to be about 20 times the expected clinical exposure level via AUC.

Study no.: 10140
Conducting laboratory and location: Toxicology Laboratories, R&D Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 17 June 1998
GLP compliance: yes, Japanese MHW
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot no. JN301, 100.2% pure

Methods

Doses: 0 and 1000 mg/kg/day b(4)
Species/strain: rat, ⌐ CD (SD), SPF
Number/sex/group: 20
Route, formulation, volume, and infusion rate: oral, in 5 ml/kg 0.5% methylcellulose solution
Study design: treatment on Day 7 to Day 17 of gestation and C-section on Day 20

Results

Mortality (dams): No deaths occurred during the study period.

Clinical signs (dams): Ptosis and lacrimation were observed in the treated group.

Body weight (dams): No treatment related effects were observed.

Food consumption (dams): No treatment related effects were observed.

Terminal and necroscopic evaluations:: No treatment related effects were observed for mean number of corpora lutea, number of implantations, number of live fetuses, implantation index, fetal mortality index, fetal sex ratio, fetal body weight or in necropsy findings in dams.

Offspring: No treatment related effects were observed.

Study title: Study by oral administration of KMD-3213 for effects on embryo- fetal development in rabbits

Key study findings: An embryo/fetal study in rabbits showed decreased maternal body weight at the high dose of 200 mg/kg/day (approximately 13-25 times the maximum recommended human exposure of parent drug via AUC). No evidence of teratogenicity was observed at this dose. Variations of lung lobation were observed at 20, 60, and 200 mg/kg/day and one fetus in each treated group (< 1%, not statistically significant) had a ventricular septal defect.

Study no.: 10050

Conducting laboratory and location: Toxicology Research Laboratory, R&D Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 8 February, 1996
GLP compliance: yes, Japanese MHW
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: Lot no. GX201,

Methods

Doses: 0, 20, 60, and 200 mg/kg/day
Species/strain: rabbit, Kbl: NZW (New Zealand White, SPF)
Number/sex/group: 20
Route, formulation, volume, and infusion rate: oral, in 5 ml/kg 0.5% methylcellulose solution
Satellite groups used for toxicokinetics: 5/group
Study design: treatment from Day 6 to Day 18 of gestation with C-section on Day 28

Results

Mortality (dams): No deaths occurred during the study period

Clinical signs (dams): Four rabbits aborted on Days 22, 25, 27, and 28 in the 200 mg/kg/day group. Emaciation was observed in these and one other dam. In the rabbit which aborted on gestation Day 25, necropsy showed a black substance adherent to the gastric mucosal surface, gastric mucosal ecchymosis and red bloody urine retention in the bladder. In the animal aborting on gestation Day 27, necropsy revealed pleural effusion, ascites and pericardial effusion. Although one control rabbit had vaginal bleeding on gestation Day 6, this animal had no abnormalities subsequently and also showed no abnormality at necropsy.

Body weight (dams): In the 20 and 60 mg/kg groups, no treatment related effects were observed. Body weight gains were reduced in the 200 mg/kg group, with a significant difference from the control group after gestation Day 7.

Food consumption (dams): In the 20 and 60 mg/kg groups, no treatment related effects were observed. In the 200 mg/kg group, food consumption was markedly reduced in some animals after the start of treatment and the mean food consumption was reduced throughout the administration period, with a significant difference from that of the control group between gestation Day 7 and Day 21.

Toxicokinetics: (from study no. 10116)

Plasma concentration and AUC in pregnant rabbits treated orally with KMD-3213

Daily Dose (mg/kg)		0 (Control)	20	60	200
Dams:					
Toxicokinetics:					
$AUC_{0\text{-}5}$ [a] (ng·hr/mL):	Day 1	Not done	145.42 ± 32.91	785.95 ± 280.60	4959.33 ± 1833.18
	Day 13 (G18)	Not done	209.37 ± 69.07	1130.88 ± 210.42	9056.41 ± 2267.86
C_{max} [a] (ng/mL):	Day 1 (G6)	Not done	59.21 ± 17.30	369.07 ± 112.91	2106.73 ± 657.80
	Day 13 (G18)	Not done	103.80 ± 41.19	562.87 ± 113.75	3879.66 ± 913.25

Mean ± S.D. G=Gestation Day.

a) Study No. 10116.

Terminal and necroscopic evaluations: No effect of treatment was observed on the number of corpora lutea, the number of implantations, the number of live fetuses, fetal sex ratio, fetal body weights or placental weights in the 20 and 60 mg/kg groups. In the 200 mg/kg group, fetal body weights and placental weights were slightly lower and embryo-fetal mortality was higher than those of control group, but no statistical significance was observed.

Group and Dose	No. of dams	No. of corpora lutea (Mean ± S.D.)	No. of implantations (Mean ± S.D.) [%, Mean± S.D.] [a]	No. of live fetuses (Mean ± S.D.)	No. of dead fetuses [%] [b]	Sex ratio (Male/Female)	Fetal body weight(g) [Mean ± S.D.] Male	Fetal body weight(g) [Mean ± S.D.] Female	Placental weight(g) [Mean± S.D.]
Control 0mg/kg	19	189 (9.9 ± 2.78)	164 (8.6 ± 3.50) [84.1 ± 20.89]	140 (7.8 ± 3.27)	15 [9.1]	0.99 (74/75)	34.6 ± 6.04	34.1 ± 7.18	5.9 ± 1.39
KMD-3213 20mg/kg	19	205 (10.8 ± 2.15)	151 (7.9 ± 4.03) [73.0 ± 32.68]	142 (7.5 ± 3.63)	9 [6.0]	1.18 (77/65)	36.7 ± 5.48	34.9 ± 5.38	5.6 ± 1.18
KMD-3213 60mg/kg	19	196 (10.3 ± 1.67)	155 (8.2 ± 3.08) [80.0 ± 27.37]	147 (7.7 ± 2.86)	8 [5.2]	0.81 (66/81)	35.8 ± 5.64	34.2 ± 7.09	5.6 ± 1.34
KMD-3213 200mg/kg	15	159 (10.6 ± 1.59)	139 (9.3 ± 1.62) [88.3 ± 14.22]	109 (7.3 ± 2.22)	30 [21.6]	0.76 (47/62)	31.5 ± 4.77	30.3 ± 7.20	5.0 ± 0.97

a): (No. of implantations / No. of corpora lutea)×100
b): (No. of dead fetuses / No. of implantations)×100

Offspring: Variations of lung lobation were observed at 20, 60, and 200 mg/kg/day and one fetus in each treated group (< 1%, not statistically significant) had a ventricular septal defect.

Group and Dose	Control 0mg/kg	KMD-3213 20mg/kg	KMD-3213 60mg/kg	KMD-3213 200mg/kg
[External anomalies]				
No. of fetuses observed	149	142	147	109
No. of fetuses with anomalies	0	0	0	0
[Visceral anomalies]				
No. of fetuses observed	149	142	147	109
No. of fetuses with anomalies(%)	0	2(1.4)	1(0.7)	1(0.9)
Types				
Abnormal origin of right subclavian artery(%)	0	1(0.7)	0	0
Persistent truncus arteriosus(%)	0	1(0.7)	1(0.7)	0
Ventricular septal defect(%)	0	1(0.7)	1(0.7)	1(0.9)
[Skeletal anomalies]				
No. of fetuses observed	149	142	147	109
No. of fetuses with anomalies(%)	3(2.0)	3(2.1)	1(0.7)	2(1.8)
Types				
Split of cervical vertebral arch(%)	0	1(0.7)	0	0
Abnormal number of cervical vertebrae(%)	0	1(0.7)	0	0
Abnormal site of thoracic vertebral arch(%)	1(0.7)	0	0	0
Deformity of thoracic vertebral arch(%)	1(0.7)	0	0	0
Fusion of thoracic vertebral centrae(%)	0	1(0.7)	0	0
Abnormal site of thoracic vertebral centra(%)	1(0.7)	1(0.7)	0	0
Deformity of thoracic vertebral centra(%)	1(0.7)	1(0.7)	0	0
Fusion of ribs(%)	2(1.3)	2(1.4)	0	1(0.9)
Fusion of thoracic and lumbar vertebral arches(%)	0	1(0.7)	0	0
Absence of lumbar vertebral arch(%)	1(0.7)	0	0	1(0.9)
Abnormal site of lumbar vertebral centra(%)	1(0.7)	1(0.7)	0	1(0.9)
Deformity of lumbar vertebral centra(%)	1(0.7)	0	0	1(0.9)
Abnormal site of caudal vertebral centra(%)	0	0	1(0.7)	0

Group and Dose	Control 0mg/kg	KMD-3213 20mg/kg	KMD-3213 60mg/kg	KMD-3213 200mg/kg
[Visceral examination]				
No. of fetuses observed	149	142	147	109
No. of fetuses with variations	0	4(2.8)*	1(0.7)	5(4.6)*
Type				
Variation of lobation of lung	0	4(2.8)*	1(0.7)	5(4.6)*
[Skeletal examination]				
No. of fetuses observed	149	142	147	109
No. of fetuses with variations(%)	110(73.8)	124(87.3)	97(66.0)	89(81.7)
Types				
Cervical rib(%)	2(1.3)	1(0.7)	1(0.7)	1(0.9)
Split of thoracic vertebral centra(%)	0	1(0.7)	0	0
Variation of number of lumbar vertebrae(%)	3(2.0)	1(0.7)	0	1(0.9)
Lumbar rib[extra](%)	91(61.1)	104(73.2)	69(46.9)	67(61.5)
Lumbar rib[rudimentary](%)	17(11.4)	20(14.1)	22(15.0)	20(18.3)
Fusion of sternebrae(%)	2(1.3)	0	7(4.8)	2(1.8)
Asymmetry of sternebrae(%)	0	5(3.5)	1(0.7)	2(1.8)
Split of sternebrae(%)	1(0.7)	5(3.5)	4(2.7)	3(2.8)
Progress of ossification				
Delayed ossification of				
Frontal(%)	1(0.7)	1(0.7)	1(0.7)	5(4.6)
Parietal(%)	1(0.7)	1(0.7)	1(0.7)	5(4.6)
Sternebrae[No. of ossification <5](%)	2(1.3)	5(3.5)	1(0.7)	5(4.6)
Sternebrae[No. of ossification <6](%)	34(22.8)	24(16.9)	29(19.7)	34(31.2)
No. of ossified metacarpal*)	4.8±0.2	4.9±0.2	4.8±0.2	4.7±0.3
No. of ossified metatarsal*)	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0
No. of ossified sacral and caudal vertebrae *)	19.5±0.4	19.7±0.5	19.5±0.3	19.6±0.7

a): Mean±S.D.
* : Significant difference from control at p<0.05

Daily Dose (mg/kg)	0 (Control)	20	60	200
Dams:				
Number of pregnant animals	19	19	19	19
Number of animals dead or sacrificed moribund	0	0	0	0
Number of animals aborted or with total resorption of litter	0	0	0	4
Clinical signs				
Emaciation	–	–	–	+
Necropsy	–	–	–	–
Body weight gain (kg) a),b)	0.25 ± 0.17	0.27 ± 0.13	0.22 ± 0.14	−0.01 ± 0.22**
Food consumption (g/day) a),b)	147.1 ± 39.8	150.8 ± 36.4	146.8 ± 51.2	61.1 ± 61.6**
Mean number of corpora lutea a)	9.9 ± 2.78	10.8 ± 2.15	10.3 ± 1.67	10.6 ± 1.59
Mean number of implantations b)	8.6 ± 3.50	7.9 ± 4.03	8.2 ± 3.08	9.3 ± 1.62
Litters:				
Number of litters evaluated	19	19	19	15
Mean number of surviving fetuses b)	7.8 ± 3.27	7.5 ± 3.63	7.7 ± 2.86	7.3 ± 2.22
Mean implantation index (%) c)	84.1 ± 20.89	73.0 ± 32.68	80.0 ± 27.37	88.3 ± 14.22
Mean postimplantation loss(%) c)	9.1	6.0	5.2	21.6
Mean fetal body weight (g) b)				
Male	34.6 ± 6.04	36.7 ± 5.48	35.8 ± 5.64	31.5 ± 4.77
Female	34.1 ± 7.18	34.9 ± 5.38	34.2 ± 7.09	30.3 ± 7.20
Fetal sex ratio (M/F) d)	0.99	1.18	0.81	0.76
Mean weight of placenta (g) b)	5.9 ± 1.39	5.6 ± 1.18	5.6 ± 1.34	5.0 ± 0.97
Fetal abnormalities:				
Number of fetus with external abnormality c)	0	0	0	0
Number of fetus with visceral abnormality c)	0	2 (1.4%)	1 (0.7%)	1 (0.9%)
Number of fetus with skeletal abnormality c)	3 (2.0%)	3 (2.1%)	1 (0.7%)	2 (1.8%)

Mean ± S.D., -: No noticeable finding, +: Finding noted, a) At the end of treatment (Gestation Day 19),

b) Dunnett's multiple comparison test (vs control): **; $p<0.01$, c) Wilcoxon's rank sum test (vs control), d) χ^2 test (vs control).

Prenatal and postnatal development

Study title: Study by oral administration of KMD-3213 for the effects on pre-and postnatal development, including maternal function in rats

Key study findings: No effects on physical or behavioral development of offspring were observed when rats were treated during pregnancy and lactation at up to 300 mg/kg/day.

Study no.: 10101 (KMD-TX1999-408E01)
Conducting laboratory and location: Toxicology Laboratories, R&D, Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 17 August 1997
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Lot # JH312, 99.8% pure

Methods b(4)

Doses: 0, 10, 30, 100 and 300 mg/kg/day
Species/strain: rat, — CD (SD), SPF
Number/sex/group: 20 females/group, age 11 weeks, 230.5-312.3 g
Route, formulation, volume, and infusion rate: oral (gavage) in 5 ml/kg 0.5% methyl cellulose solution
Study design: Dosing was from Day 7 of gestation through Day 20 of lactation

Results

F_0 in-life: In the 300 mg/kg dose group, 1, 4 and 1 dams died on days 21, 22 and 23 of gestation, respectively. Three of the dams died during delivery on days 22 or 23 of gestation. Ptosis was observed in all the KMD-3213 dosed groups on day 7 of gestation and after. Lacrimation was observed on day 9 of gestation in the 300 mg/kg dose group. Lacrimation was also observed in the late gestation period in the 10, 30 and 300 mg/kg dose pups. The above findings were observed also in the morning following treatment in a few dams. In one dam treated with 300 mg/kg, mucous stool was observed on day 22 of gestation. No dams died during lactation. Ptosis was observed in a few dams treated with 30 and 300 mg/kg on days 0 to 2 of lactation. A dam in the 300 mg/kg dose group had staining around the eyes on day 2 of lactation. No treatment related effects on body weight were observed. In the 300 mg/kg dose group, there was one dam with abnormal delivery that had been confirmed to be littering in the afternoon on day 22 of gestation, but had fetuses in the abdominal cavity in the morning on day 24 of gestation. In the 100 mg/kg dose group, there was one dam which had been noted to have vaginal bleeding from the morning through the afternoon of day 23 of gestation, but the presence of pups could not be confirmed even on day 24 of gestation. Total litter loss was observed in 2 dams (on days 1 and 2 postpartum), 1 dam (on day 3 postpartum) and 2 dams (on days 1 and 3 postpartum) in the control, 30 and 300 mg/kg dose groups, respectively. At

parturition and during lactation, poor postpartum nursing and poor nest building were observed incidentally in the control, 30, 100 and 300 mg/kg dose groups. Although no effects on body weight of dams were observed, slight effects of food consumption during the lactation period were observed.

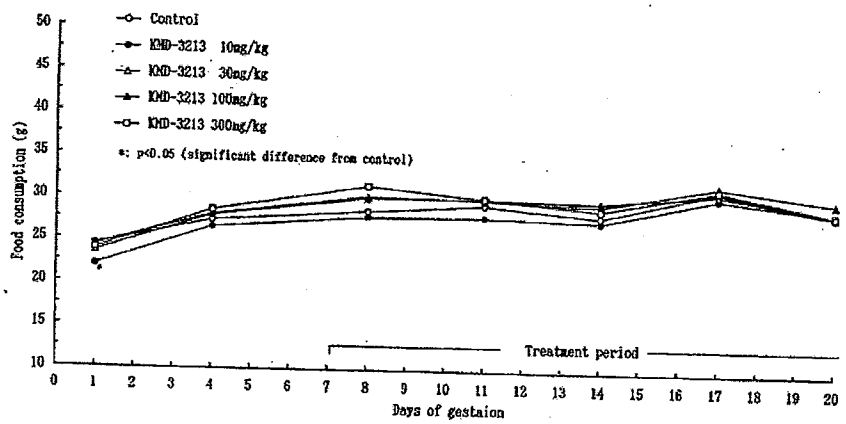
Daily Dose (mg/kg)	0 (Control)	10	30	100	300
F_0 Females:					
Number of pregnant animals	20	20	20	19	20
Clinical signs					
Eyelid ptosis	-	+	+	+	+
Lacrimation	-	+	+	-	+
Necropsy					
Stomach; Bleeding lesion	-	-	-	-	+ [a]
Body weight gain during gestation (g) [b,c]	170.5 ± 27.7	174.9 ± 18.7	172.3 ± 17.5	169.7 ± 31.2	185.1 ± 16.6
Body weight gain during lactation (g) [b,c]	48.9 ± 21.5	50.2 ± 11.0	46.6 ± 12.7	44.5 ± 16.1	44.4 ± 22.5
Food consumption during gestation (g) [b,c]	28.6 ± 4.0	28.8 ± 2.7	28.7 ± 2.9	30.1 ± 3.3	28.8 ± 2.5
Food consumption during lactation (g) [b,c]	85.5 ± 10.5	81.9 ± 7.3	80.8 ± 7.7	76.5 ± 10.0*	76.0 ± 13.9*
Mean duration of gestation (days) [c]	22.2 ± 0.4	22.0 ± 0.5	21.9 ± 0.3	21.8 ± 0.4 [d]	21.9 ± 0.5
Abnormal parturition	0	0	0	0	1 [e]

Mean ± S.D.

-: No noticeable finding, +:Finding noted.

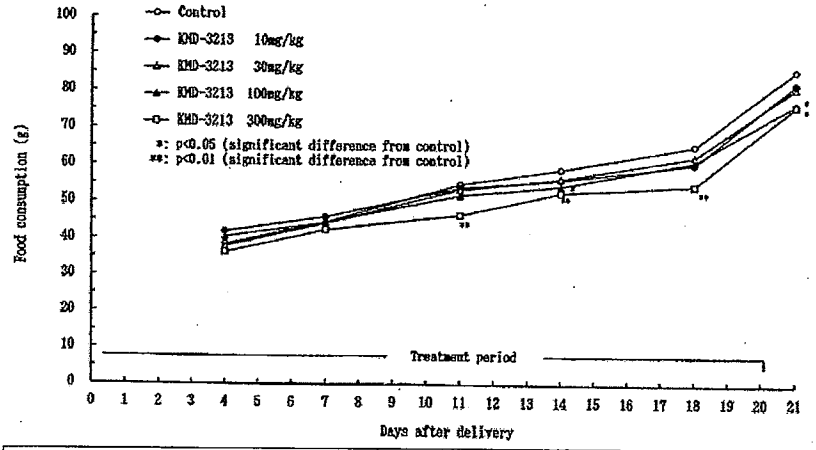
a) Observed in all of dead animals, b) At the final point during gestation or lactation period, c) Dunnett's multiple comparison test (vs control); *: $p<0.05$, d) Except for one dam for which no birth offspring was confirmed, e) A dam that had not yet completed delivery by Gestation Day 24.

Food consumption by dams during the gestation period:



Food consumption by dams during the lactation period:

APPEARS THIS WAY
ON ORIGINAL



Daily Dose (mg/kg)	0 (Control)	10	30	100	300
F_1 Litters (before weaning): Number of litters evaluated	20	20	20	19	13
Mean number of implantations [a]	16.6 ± 2.6	16.5 ± 1.9	16.8 ± 2.4	16.1 ± 5.5	17.7 ± 1.6
Mean number of live-born pups/litter [a]	13.6 ± 3.5	14.9 ± 2.2	14.9 ± 2.4	14.1 ± 4.9	13.3 ± 4.7
Mean stillborn rate (%) [b]	9.1 ± 20.7	3.8 ± 7.3	3.9 ± 8.6	3.0 ± 8.2	16.7 ± 24.5
Delivery index (%) [c]	100.0	100.0	100.0	94.7	100.0
Birth index (%) [b]	82.6 ± 19.6	90.4 ± 9.1	89.1 ± 10.4	84.3 ± 22.8	75.1 ± 26.0
Postnatal survival rate on Day 4 after birth (%) [b]	87.0 ± 30.9	97.3 ± 6.4	91.7 ± 22.2	98.3 ± 2.9	79.0 ± 39.5
Postnatal survival rate to weaning (%) [b]	99.3 ± 2.9	100.0 ± 0.0	100.0 ± 0.0	99.3 ± 2.9	100.0 ± 0.0
Birth body weight (g) [a] Male	6.6 ± 0.7	6.4 ± 0.5	6.5 ± 0.6	6.6 ± 0.7	6.3 ± 0.3
Female	6.2 ± 0.6	6.2 ± 0.8	6.1 ± 0.5	6.2 ± 0.6	6.0 ± 0.3
Pup sex ratios at birth (M/F) [c]	1.01	0.93	1.14	0.91	1.06
Clinical signs of offspring	–	–	–	–	–
Necropsy of offspring	–	–	–	–	–
Physical development [b,c]	–	–	–	–	–

Mean ± S.D.

-: No noticeable finding.

a) Dunnett's multiple comparison test (vs control), b) Wilcoxon's rank sum test (vs control), c) χ^2 test (vs control).

Daily Dose (mg/kg)	0 (Control)	10	30	100	300
F_1 Male (after weaning): Number of litter evaluated after weaning per litter	18	20	19	18	11 [e]
Number of animals dead or sacrificed moribund	0	0	0	0	0
Clinical signs	–	–	–	–	–
Necropsy	–	–	–	–	–
Body weight [d] (g) [a]	469.5 ± 29.4	487.9 ± 30.8	473.4 ± 27.9	482.4 ± 44.3	469.2 ± 27.7
Preputial separation [c]	–	–	–	–	–
Sensory function [b]	–	–	–	–	–
Motor activity [b]	–	–	–	–	–
Learning and memory [b]	–	–	–	–	–
Mean number of days prior to mating [a]	2.6 ± 1.9	3.2 ± 1.7	3.5 ± 2.4	3.3 ± 2.0	4.4 ± 4.2
Number of males that mated	17	17	15	18	10
Number of fertile males	16	16	13	17	9
Copulation index (%) [c]	94.4	85.0	78.9	100.0	100.0
Fertility index (%) [c]	94.1	94.1	86.7	94.4	90.0

Mean ± S.D.

-: No noticeable finding.

a) Dunnett's multiple comparison test (vs control), b) Wilcoxon's rank sum test (vs control), c) χ^2 test (vs control).

d) At the final point before mating period (Day 70 of birth), e) Reproductive function was evaluated using animals from 10 litters.

Daily Dose (mg/kg)	0 (Control)	10	30	100	300
F_1 Female (after weaning):					
Number of litter evaluated after weaning per litter	18	20	19	18	10
Number of animals dead or sacrificed moribund	0	0	0	0	0
Clinical signs	–	–	–	–	–
Necropsy	–	–	–	–	–
Body weight before mating [a] (g) [c]	291.8 ± 25.8	294.6 ± 32.5	285.8 ± 30.9	288.4 ± 30.6	294.1 ± 25.5
Body weight gain during gestation [b] (g) [c]	93.6 ± 16.5	92.1 ± 12.1	92.7 ± 11.6	96.1 ± 28.7	99.0 ± 15.5
Food consumption during gestation [b] (g) [c]	30.3 ± 4.2	28.0 ± 2.7	29.5 ± 2.7	30.2 ± 6.6	31.6 ± 4.4
Opening of vagina [d]	–	–	–	–	–
Sensory function [e]	–	–	–	–	–
Motor activity [e]	–	–	–	–	–
Learning and memory [e]	–	–	–	–	–
Mean number of days prior to mating [c]	2.6 ± 1.9	3.2 ± 1.7	3.5 ± 2.4	3.3 ± 2.0	4.4 ± 4.2
Number of fertile animals	16	16	13	17	9
Mean number of corpora lutea [c]	16.6 ± 2.63	17.9 ± 1.98	17.3 ± 1.75	17.6 ± 1.33	18.0 ± 2.55
Mean number of implantation [c]	14.4 ± 4.94	16.6 ± 1.46	16.0 ± 2.12	17.0 ± 1.27	17.1 ± 2.52
Mean implantation rate (%) [e]	83.6 ± 24.91	92.9 ± 8.43	92.3 ± 7.04	96.7 ± 3.86	95.1 ± 5.24
Copulation index (%) [d]	94.4	85.0	78.9	100.0	100.0
Fertility index (%) [d]	94.1	94.1	86.7	94.4	90.0
F_2 litters:					
Mean number of surviving embryo/litter [c]	12.9 ± 5.77	15.3 ± 1.65	15.0 ± 1.83	16.1 ± 2.16	16.1 ± 2.42
Mean postimplantation loss(%) [e]	10.5 ± 21.7	8.0 ± 4.8	5.9 ± 7.1	5.8 ± 8.8	5.7 ± 5.9

Mean ± S.D.

-: No noticeable finding.

a) At the final point before mating (Day 70 of birth), b) At the final point of gestation period,

c) Dunnett's multiple comparison test (vs control), d) χ^2 test (vs control), e) Wilcoxon's rank sum test (vs control).

F_0 necropsy:

	Females (mg/kg/day)				
	0	10	30	100	300
Number of dams observed/pregnant	20	20	20	19	20
Died during gestation to delivery	0	0	0	0	6
__stomach, focal hemorrhage					6
__stomach, erosion					2
__stomach/cecum, watery contents					2
__liver, congestion					3
__liver, white spots					1
__adrenal gland, slight swelling					1
__kidney, congestion					1
Sacrificed at delivery	0	0	0	1	1
__uterus, wound (conceptus in abdomen)					1
Sacrificed during lactation period	2	0	1	0	2
__mammary gland, slight immaturity			1		1
Number of delivered dams	20	20	20	19	13
Number of liveborns (average % / litter)	13.6	14.9	14.9	14.1	13.3
No. of implantations (average % / litter)	16.6	16.5	16.8	16.1	17.7
Birth index (liveborn/implantations) x100	82.6	90.4	89.1	84.3	75.1

F_1 physical development: No treatment related effects on survival, body weight, abnormalities, pinna detachment, eruption of incisors, separation of eyelids, vaginal opening, or cleavage of balanopreputial gland were observed. One fetus with anury and anal atresia was observed in 10 mg/kg dose group. Although slightly lower values for the 4-day viability index were observed in the 300 mg/kg dose group, the difference was not statistically significant.

F_1 behavioral evaluation: N=4 animals/sex/litter

Open-field performance	F1 males (mg/kg/day)				
	0	10	30	100	300
Ambulation	35.6	38.0	33.9	40.6	50.3*
Rearing	8.5	12.1	8.0	8.4	10.0
Grooming	0.1	0.3	0.3	0.2	0.4
Defecation	0.9	1.0	1.2	1.1	0.5
Urination	1.3	1.2	2.1	1.8	2.1

Open-field performance	F1 females (mg/kg/day)				
	0	10	30	100	300
Ambulation	39.9	44.4	40.5	52.8	47.2
Rearing	13.7	14.3	11.9	17.4	13.9
Grooming	1.2	0.2	0.2	0.6	0.2
Defecation	0.5	0.4	0.5	0.6	0.3
Urination	0.9	1.2	1.3	1.0	1.1

F_1 reproduction: No treatment related effects were observed.

2.6.6.7 Local tolerance

KMD-3213: Intramuscular local irritation study in rabbits (2002IF005, 13 February 2002, GLP). KMD-3213 was injected into the vastus lateralis muscle of rabbits. The local irritation was compared with those of physiological saline and 0.425 and 1.7 vol% acetic acid by gross and histopathological examinations on Days 2 and 14.

On day 2, very slight or moderate hemorrhage and very slight or slight white discoloration, along with very slight or slight necrosis of the muscular fibers, was observed in the muscles treated with KMD-3213. Slight hemorrhage and slight or moderate white discoloration, along with moderate or severe necrosis of the muscular fibers, was observed in the muscles treated with 0.425 vol% acetic acid. Nearly complete recovery of gross and histopathological lesions was evident on day 4 for both treatments. No abnormalities were evident on day 14 for injection of physiological saline. The local irritation of KMD-3213 was classified as Grade 2: local irritation less severe than that of 0.425 vol% acetic acid on both Days 2 and 14, but more severe than that of physiological saline on either day.

2.6.6.8 Special toxicology studies

***In vitro* hemolysis study with human whole blood (2002IF006, 2002, GLP).** At a concentration of 0.2 mg/ml in peripheral blood from healthy male volunteers, no hemolytic potential was detected, as measured by discoloration, turbidity, or hemolysis rate.

***In vitro* study of inhibitory effect on trypsin.**
Report title: *In Vitro* Trypsin Inhibition Study of KMD-3213 on Trypsin
Enzyme type: Trypsin (derived from bovine pancreas) Study No. 10281
Base: Ac-Arg-pNA Test Article: Silodosin
Vehicle: 0.1M physiological saline solution of citric acid
GLP Compliance: Yes
Positive control: Monohydrate of hemisulfate of leupeptin
Date of Treatment: September 19, 2002
Special Features: None

Test substance	Dose (μ g/mL)	% of Control	IC50 (μ g/mL)
Control	0	100	-
silodosin	0.001	102	
	0.003	109	
	0.01	105	
	0.03	110	
	0.1	112	
	0.3	111	
	1	117	
	3	120	
	10	134	

– : Not calculated.

***In vitro* study of inhibitory effect on papain.**
Report title: *In Vitro* Papain Inhibition Study of KMD-3213 Study No. 10282
Enzyme type: Papain (derived from papaya latex) Test Article: Silodosin
Base: Ac-Phe-Gly-pNA GLP Compliance: Yes
Vehicle: 0.1M physiological saline solution of citric acid
Date of treatment: September 20, 2002
Positive control: Monohydrate of hemisulfate of leupeptin
Special Feature: None

Test substance	Dose (μ g/mL)	% of Control	IC_{50} (μ g/mL)
Control	0	100	-
silodosin	0.001	100	
	0.003	100	
	0.01	101	
	0.03	100	
	0.1	99.3	
	0.3	100	
	1	99.3	

	3	100	
	10	100	

Test substance	Dose (μ g/mL)	% of Control	IC_{50} (μ g/mL)
leupeptin	0.001	95.7	
	0.003	91.4	
	0.01	76.1	
	0.03	40.2	
	0.1	7.64	0.0245
	0.3	3.16	
	1	1.66	
	3	1.33	
	10	1.16	
Blank	0	–	–

Study title: Silodosin: Evaluation of *in vitro* phototoxicity on Balb/c 3T3 fibroblasts using neutral red assay.

Key study findings: A small increase in phototoxicity over control (classified as a "probable" level of phototoxicity) was observed in the presence of silodosin.

Study no.: 2720/1-D6173
Conducting laboratory and location: b(4)

Date of study initiation: May, 2007
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: Silodosin KMD-3213, batch number T02090505, 100.1% pure
Formulation/vehicle: 1% DMSO in PBS

Methods

Silodosin was assayed in the Neutral Red uptake assay in the presence and absence of UV-A light. Chlorpromazine was used as a positive control. The cultures were treated for 1 hour at 37°C prior to irradiation. One set of plates were exposed to 5 J/cm2 UV-A and a second set of plates were kept in the dark for the same period. After irradiation, the media was aspirated from each well, cells washed with a suitable volume of PBS, and finally 0.2 ml medium was added to each well. The plates were then incubated for 20 ± 2 hours at 37°C in a humidified atmosphere of 5% CO2 in air.

Doses:
Experiments 1, 3 and 4:
Silodosin; +UV-A: 0.3160, 1.000, 3.160, 10.00, 31.60, 100.0, 316.0, 1000 μg/ml
Silodosin; -UV-A: 0.3160, 1.000, 3.160, 10.00, 31.60, 100.0, 316.0, 1000 μg/ml

Experiment 2:
Silodosin; +UV-A: 49.87, 69.32, 96.36, 133.9, 186.0, 258.8, 359.7, 500.0 µg/ml
Silodosin; -UV-A: 49.87, 69.32, 96.36, 133.9, 186.0, 258.8, 359.7, 500.0 µg/ml

Experiments 1-4:
CPZ, +UV-A: 0.1, 1, 10, 100 µg/ml
CPZ, -UV-A: 1, 10, 100, 1000 µg/ml

Results: A small increase in phototoxicity over control (classified as a "probable" level of phototoxicity) was observed in the presence of silodosin.

Experiment	Test article	IC_{50} absence of UV-A (µg/mL)	IC_{50} presence of UV-A (µg/mL)	PIF Value
1	Silodosin	319.789	144.885	2.207
	Chlorpromazine	45.802	0.980	46.737**
2	Silodosin	*	66.023	> 7.573#
	Chlorpromazine	55.675	1.662	33.499**
3	Silodosin	263.145	73.801	3.567
	Chlorpromazine	59.497	0.976	60.960**
4	Silodosin	396.292	99.670	3.976
	Chlorpromazine	40.902	1.444	28.325**

* Value could not be calculated
\# Estimated PIF
** PIF ≥ 6, therefore positive control response was acceptable.

Study title: Single dose oral (gavage) phototoxicity evaluation of silodosin in hairless mice

Key study findings: In a single dose oral (gavage) phototoxicity evaluation of silodosin in hairless mice (N=6), only mild erythema was observed after 4 hours simulated sunlight exposure at high silodosin exposure levels.

Study no.: ONY00026
Conducting laboratory and location: b(4)

Date of study initiation: 6 May 2008
GLP compliance: yes
QA reports: yes (x) no ( )
Drug, lot #, and % purity: silodosin KMD-3213, lot number KMD060781, 99.9 % pure

Methods: In male and female — SKH1-*hr* hairless mice the following doses were administered; 8-Methoxypsoralen (8 MOP) was administered as a positive control. b(4)

Male Mice

Group	Formulation					UVR Exposure	
	Descriptor	Dosage (mg/kg)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Route	Instrumental UVR Dose (MED)	Interval Between Formulation Administration and UVR Exposure (Hours)
1	0 (Vehicle)	0 (Vehicle)	0	5	oral	0.5	4 ± 15 minutes
2	silodosin	20	4	5	oral	0.5	4 ± 15 minutes
3	silodosin	100	20	5	oral	0.5	4 ± 15 minutes
4	silodosin	500	100	5	oral	0.5	4 ± 15 minutes
5	8-MOP	50	5	10	oral	0.5	1 ± 15 minutes

MED –Minimal Erythema Dose (a UVR dose adequate to elicit a barely perceptible response in skin).

Female Mice

Group	Formulation					UVR Exposure	
	Descriptor	Dosage (mg/kg)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Route	Instrumental UVR Dose (MED)	Interval Between Formulation Administration and UVR Exposure (Hours)
1	0 (Vehicle)	0 (Vehicle)	0	5	oral	0.5	4 ± 15 minutes
2	silodosin	60	12	5	oral	0.5	4 ± 15 minutes
3	silodosin	150	30	5	oral	0.5	4 ± 15 minutes
4	silodosin	400	80	5	oral	0.5	4 ± 15 minutes
5	8-MOP	50	5	10	oral	0.5	1 ± 15 minutes

MED –Minimal Erythema Dose (a UVR dose adequate to elicit a barely perceptible response in skin).

The mice were administered formulations orally via gavage once. For UVR exposure, all mice were lightly anesthetized via intraperitoneal injection of chloral hydrate in deionized water, 60 mg/mL, approximately 0.15 mL/mouse, and then positioned on plastic tubing with laboratory tape. An aluminum foil mask was placed over each animal before UVR exposure. The mask had a single hole with a diameter of approximately 1.3 cm (1.3 cm^2). The hole in the mask was placed over the mid-dorsal area. UVR exposure began approximately 4 hours after completion of formulation administration for Groups 1 through 4 and approximately 1 hour after completion of formulation administration for Group 5. The UVR exposure duration was 30 ± 5 minutes for all groups.

The source of irradiation, a 6.5 kw long-arc xenon water-cooled lamp, was used to simulate mid-latitude summer sunlight. One filter [redacted] 1 mm thick) was used to attenuate mid-range ultraviolet radiation (UVB), and an instrumental dosage of about 0.5 MED was delivered.

b(4)

Checks for viability were conducted at least twice daily. Clinical observations were performed at least weekly during acclimation and at least once before formulation administration. Mice were monitored constantly until recovery from anesthesia. Mice were individually examined immediately after formulation administration, approximately 60 minutes, 4 hours, 1, 2 and 3 days after the completion of UVR exposure for general appearance and signs of skin responses. Body weights were recorded at least weekly during acclimation and daily on Days 1 through 4. All mice were sacrificed and discarded without further evaluation at the completion of the study.

Results:

In male mice, a single oral administration of silodosin at a dosage of 500 mg/kg elicited cutaneous reactions indicative of phototoxicity after simulated sunlight exposure. In male mice, a single oral administration of silodosin at dosages of 0 (Vehicle), 20 and 100 mg/kg did not elicit skin reactions indicative of phototoxicity. At 4 hours after simulated sunlight exposure in male mice administered the test article formulations, erythema grade 1 (barely perceptible light redness) occurred in 1 and 3 mice administered the 100 and 500 mg/kg test article dosages. Erythema in a single male mouse in the 500 mg/kg dosage group persisted at one day after light exposure. Excessive hair growth, a common observation in this test system, occurred in a single male mouse in each of Groups 2 and 3. No other skin reactions occurred in male mice administered the test article formulations.

At 4 hours after simulated sunlight exposure in female mice administered a single dose of test article, erythema grade 1 occurred in 1, 2, 2 and 5 mice administered the 0 (Vehicle), 60, 150 and 400 mg/kg test article dosages, respectively. Erythema in a single female mouse in the 400 mg/kg dosage group persisted at one day after light exposure. Erythema in the mice in the 400 mg/kg dosage group: 1) was dosage-dependent; 2) affected 5 of 6 mice, and 3) the skin reaction persisted into the day following light exposure in one mouse. Erythema in 2 female mice in each of the 60 and 150 mg/kg test article dosage groups 1) affected only 2 mice in each group; and 2) was transient. Erythema in a mouse in the vehicle-treated group was limited to a single mouse and was transient. Excessive hair growth, a common observation in this test system, occurred in a single female mouse in each of Groups 3, 4 and 5. No other skin reactions occurred in female mice administered the test article formulations.

In mice orally administered the comparator article, 8-MOP, skin reactions indicative of phototoxicity occurred in all mice and included erythema, edema and scab.

SKIN REACTION AND CLINICAL OBSERVATIONS – SUMMARY – MALE MICE

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	20	100	500	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	1c	0	0	0	0
DAYS 1 THROUGH 4 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
ERYTHEMA: GRADE 1	0/ 0	0/ 0	1/ 1	4/ 3	15/ 6
SCAB	0/ 0	0/ 0	0/ 0	0/ 0	10/ 6
EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	8/ 6
CLINICAL OBSERVATIONS:					
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	4/ 1	4/ 1	0/ 0	0/ 0
DEHYDRATION - MODERATE	1/ 1c	0/ 0	0/ 0	0/ 0	0/ 0
RIGHT FLANK: LUMP(S)	1/ 1c	0/ 0	0/ 0	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1401 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	20	100	500	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	1c	0	0	0	0
DAY 1 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
1 HOUR FOLLOWING UVR EXPOSURE	NO ADVERSE FINDINGS				
4 HOURS FOLLOWING UVR EXPOSURE:					
SITE 1:					
ERYTHEMA: GRADE 1	0/ 0	0/ 0	1/ 1	3/ 3	6/ 6
CLINICAL OBSERVATIONS:					
IMMEDIATELY FOLLOWING FORMULATION DOSAGE.					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	1/ 1	1/ 1	0/ 0	0/ 0
1 HOUR FOLLOWING UVR EXPOSURE					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	1/ 1	1/ 1	0/ 0	0/ 0
COLD TO TOUCH	0/ 0	0/ 0	0/ 0	1/ 1	0/ 0
4 HOURS FOLLOWING UVR EXPOSURE:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	1/ 1	1/ 1	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1401 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	20	100	500	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	1c	0	0	0	0
DAY 2 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	6/ 6
ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	1/ 1	3/ 3
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	1/ 1	1/ 1	0/ 0	0/ 0
DEHYDRATION - MODERATE	1/ 1c	0/ 0	0/ 0	0/ 0	0/ 0
RIGHT FLANK: LUMP(S)	1/ 1c	0/ 0	0/ 0	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1401 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	20	100	500	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	1c	0	0	0	0
DAY 3 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	4/ 4
EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	2/ 2
SCAB	0/ 0	0/ 0	0/ 0	0/ 0	4/ 4
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	1/ 1	1/ 1	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1401 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	20	100	500	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	1c	0	0	0	0
DAY 4 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
SCAB	0/ 0	0/ 0	0/ 0	0/ 0	6/ 6
ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	2/ 2
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	1/ 1	1/ 1	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1401 was sacrificed on day 2 of study due to adverse clinical observations.

SKIN REACTION AND CLINICAL OBSERVATIONS SUMMARY, FEMALE MICE

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	60	150	400	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	0	1c	0	0	0
DAYS 1 THROUGH 4 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
SCAB	0/ 0	0/ 0	0/ 0	0/ 0	12/ 6
ERYTHEMA: GRADE 1	1/ 1	2/ 2	2/ 2	6/ 5	10/ 6
EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3
GRADE 2	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	4/ 1	4/ 1	1/ 1
COLD TO TOUCH	1/ 1	1/ 1c	1/ 1	0/ 0	0/ 0
LOWER MIDLINE: PURPLE	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0
DEHYDRATION - MILD	0/ 0	1/ 1c	0/ 0	0/ 0	0/ 0
RIGHT FLANK: LUMP(S)	0/ 0	1/ 1c	0/ 0	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1440 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP	1	2	3	4	5
DESCRIPTOR	VEHICLE	SILODOSIN	SILODOSIN	SILODOSIN	8-MOP
DOSAGE (MG/KG)a	0 (VEHICLE)	60	150	400	50
UVR EXPOSURE (MED)	0.5	0.5	0.5	0.5	0.5
INTERVALS (HOURS)b	4	4	4	4	1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	0	1c	0	0	0
DAY 1 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
1 HOUR FOLLOWING UVR EXPOSURE:		NO ADVERSE FINDINGS			
4 HOURS FOLLOWING UVR EXPOSURE:					
SITE 1:					
ERYTHEMA: GRADE 1	1/ 1	2/ 2	2/ 2	5/ 5	6/ 6
CLINICAL OBSERVATIONS:					
IMMEDIATELY FOLLOWING FORMULATION DOSAGE					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	1/ 1	1/ 1	1/ 1
1 HOUR FOLLOWING UVR EXPOSURE:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	1/ 1	0/ 0	1/ 1
COLD TO TOUCH	1/ 1	1/ 1c	1/ 1	0/ 0	0/ 0
4 HOURS FOLLOWING UVR EXPOSURE:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	1/ 1	1/ 1	1/ 1
LOWER MIDLINE: PURPLE	0/ 0	0/ 0	1/ 1	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1440 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP DESCRIPTOR DOSAGE (MG/KG)a UVR EXPOSURE (MED) INTERVALS (HOURS)b	1 VEHICLE 0 (VEHICLE) 0.5 4	2 SILODOSIN 60 0.5 4	3 SILODOSIN 150 0.5 4	4 SILODOSIN 400 0.5 4	5 8-MOP 50 0.5 1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	0	1c	0	0	0
DAY 2 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	1/ 1	3/ 3
EDEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3
GRADE 2	0/ 0	0/ 0	0/ 0	0/ 0	3/ 3
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	1/ 1	1/ 1	0/ 0
DEHYDRATION - MILD	0/ 0	1/ 1c	0/ 0	0/ 0	0/ 0
RIGHT FLANK: LUMP(S)	0/ 0	1/ 1c	0/ 0	0/ 0	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1440 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP DESCRIPTOR DOSAGE (MG/KG)a UVR EXPOSURE (MED) INTERVALS (HOURS)b	1 VEHICLE 0 (VEHICLE) 0.5 4	2 SILODOSIN 60 0.5 4	3 SILODOSIN 150 0.5 4	4 SILODOSIN 400 0.5 4	5 8-MOP 50 0.5 1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	0	1c	0	0	0
DAY 3 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
ERYTHEMA: GRADE 1	0/ 0	0/ 0	0/ 0	0/ 0	1/ 1
SCAB	0/ 0	0/ 0	0/ 0	0/ 0	6/ 6
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	1/ 1	1/ 1	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1440 was sacrificed on day 2 of study due to adverse clinical observations.

GROUP DESCRIPTOR DOSAGE (MG/KG)a UVR EXPOSURE (MED) INTERVALS (HOURS)b	1 VEHICLE 0 (VEHICLE) 0.5 4	2 SILODOSIN 60 0.5 4	3 SILODOSIN 150 0.5 4	4 SILODOSIN 400 0.5 4	5 8-MOP 50 0.5 1
MICE TESTED	6	6	6	6	6
UNSCHEDULED SACRIFICE	0	1c	0	0	0
DAY 4 OF STUDY:					
SKIN REACTION OBSERVATIONS:					
SITE 1:					
SCAB	0/ 0	0/ 0	0/ 0	0/ 0	6/ 6
CLINICAL OBSERVATIONS:					
WHOLE BODY: EXCESSIVE HAIR GROWTH	0/ 0	0/ 0	1/ 1	1/ 1	0/ 0

N/N = TOTAL NUMBER OF OBSERVATIONS/NUMBER OF MICE WITH OBSERVATION
MED = MINIMAL ERYTHEMA DOSE 8-MOP = 8-METHOXYPSORALEN
a. Formulation administration and UVR exposure occurred on day 1 of study.
b. Approximate interval between formulation administration and UVR exposure.
c. Mouse 1440 was sacrificed on day 2 of study due to adverse clinical observations.

2.6.6.9 Discussion and Conclusions

In a 26 week study in rats, at 15 mg/kg/day (approximately equal to the expected clinical exposure via AUC), pharmacological signs included ptosis, lacrimation, and salivation. Slight to moderate fatty degeneration of hepatocytes (males) and slight swelling of centrilobular hepatocytes (males) were also observed. Increased lipid droplets were observed in the liver by electron microscopy in males. At 60 mg/kg/day (about 5 – 7 times the expected clinical exposure), discoloration of the liver was observed. Histopathology included slight to moderate fatty degeneration of hepatocytes (males), slight swelling of centrilobular hepatocytes (males), slight eosinophilic changes of centrilobular hepatocytes (males), slight dilatation of the adrenal cortex (males), hypertrophy of the vaginal mucous epithelium, and slight mammary gland hyperplasia (females). Increased lipid droplets were observed in the liver by electron microscopy in males. At 300 mg/kg/day (estimated 20 times the expected clinical exposure), clinical signs also included deep respiration and decrease in locomotor activity. Increased relative liver and relative adrenal weights and decreased uterine weight were observed. Slight fatty degeneration of hepatocytes was observed in females and moderate to severe fatty degeneration was observed in males. Histopathology included slight swelling of centrilobular hepatocytes (males and females), slight eosinophilic changes of centrilobular hepatocytes (males and females), slight dilatation of the adrenal cortex (males), hypertrophy of the vaginal mucous epithelium, and slight to moderate mammary gland hyperplasia (females) with increased secretory activity. Increased cytochrome p450 content was observed in the liver at this dose and was higher in males than in females (p 450s were not measured at 60 mg/kg/day). Proliferation of the smooth surfaced endoplasmic reticulum was observed by electron microscopy, in the liver but not the kidney. Increased lipid droplets were observed in the liver by electron microscopy in males and females. In an additional 26 week study in rats, at 0, 1, and 5 mg/kg/day, slight fatty degeneration of hepatocytes was observed. No other effects were observed in this study.

In a 52 week oral dose study in dogs, 20 mg/kg/day (about 12 -19 times the expected clinical exposure via AUC) was a No Observed Adverse Effect Level (NoAEL). Although pharmacological signs were observed at this dose (and at 5 mg/kg/day), their severity was decreased by Week 3. Brown discoloration was observed in liver and kidney at all treated doses, and liver tissue stained slightly positive for neutral lipids. At 80 mg/kg/day (about 51 – 118 times), pharmacological signs were observed for the duration of the study (52 weeks), for several hours following administration. Decreased body weights/body weight gain and decreased hemoglobin were observed. Liver tissue stained positive (slight to moderate) for neutral lipids. No indication of tissue damage was observed, but an apparent increase in alkaline phosphatase was observed (without statistical significance).

In dogs and rats, liver hypertrophy is commonly seen due to a proliferation of cytochrome p450s and is usually not considered relevant to clinical use if no signal is

observed in the clinic. Accumulation of lipid and discoloration of the liver was not accompanied by toxicity in dogs, but slight fatty degeneration of hepatocytes was observed in rats at all doses tested, including the control. No clearly drug related hepatic effects were observed in clinical studies, but clinical monitoring of liver effects will continue into phase IV of development.

In a 13 week oral dose study in dogs a NoAEL for delayed maturation of testes and epididymis and absence of sperm was 10 mg/kg/day. At 50 mg/kg/day, these effects were observed in the 13 week study; however, they were not apparent at termination of the 80 mg/kg/day group in the 52 week study. It may be speculated that differences between these studies reflect the different maturation levels of the dogs at the time of termination.

In a two-week intravenous toxicity study of the major human glucuronidated metabolite, MD127K (KMD-3213), it was found to be similar both in pharmacology and toxicology to the parent drug, silodosin. Pharmacology studies showed this metabolite to be slightly less active than the parent drug, and distribution studies in rats showed it to be distributed to tissue, including the prostate.

Neither silodosin nor its glucuronidated metabolite increased the number of revertant colonies at any dose tested, and both were judged to be not mutagenic in bacterial mutation assays.

Increases in mutant frequency were not observed at any dose of silodosin tested, and it was concluded that it was not genotoxic under the conditions tested in a mammalian cell mutation assay

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In Chinese hamster lung fibroblast cells, no increase in chromosomal aberrations were observed at any dose of silodosin tested by the 24- or 48-hour direct method or by the 6-hour treatment activation method in the presence of S-9. However, in the 6-hour treatment in the absence of S-9, chromosomal aberrations were observed and confirmed in an additional assay. Although mitotic index was not measured in this study, an additional study was also performed, in which decreased mitotic index (toxicity) was found to be associated with chromosomal aberrations under similar conditions at similar concentrations. Chromosomal aberrations in cell culture at high, cytotoxic doses, are not expected to be relevant to clinical use.

The glucuronide metabolite of silodosin was found to be not mutagenic under the conditions of a chromosomal aberration assay in cultured Chinese hamster cells.

No increase in micronuclei was observed in mice at doses up to 1000 mg/kg silodosin, and it was judged to be not genotoxic under the conditions of this assay.

In a rat liver DNA repair (UDS) test, silodosin did not cause any significant increases in either the gross nuclear grain count or the net nuclear grain count (i.e. the gross nuclear pain count minus the cytoplasmic grain count) at any dose level at either sampling time, and was therefore judged to be not genotoxic under the conditions of this assay.

In a carcinogenicity study by dietary administration of silodosin to CD-1 mice for 104 weeks at doses up to 100 mg/kg/day (about 19 times the exposure of the maximum recommended human dose or MHRE via AUC) in males and 400 mg/kg/day in females (about 68 times the MRHE via AUC), there were no significant tumor findings in male mice. Female mice treated for 2 years with doses of 150 mg/kg/day (about 29 times the MRHE via AUC) or greater had statistically significant increases in the incidence of mammary gland adenoacanthoma and adenocarcinomas, associated with hyperprolactinemia. Mice do not produce glucuronidated silodosin, which is present in human serum at approximately 4 times the level of circulating silodosin. In an additional carcinogenicity study by dietary administration to male CD-1 mice for 104 weeks (replacement study for male mice killed in excessive numbers through fighting during the previous 2-year assay), the study was negative for drug related neoplasms. Mice do not produce the major human glucuronidated metabolite.

In a 2-year oral carcinogenicity study in rats administered doses up to 150 mg/kg/day (about 8 times the exposure of the maximum recommended human dose or MHRE via AUC of silodosin), an increase in thyroid follicular cell tumor incidence was seen in male rats receiving doses of 150 mg/kg, along with increased metabolism of and decreased circulating levels of thyroxine (T4). Although the incidence of follicular cell adenomas in female rats was increased, the incidences in dosed groups were not statistically significant. There was increased incidence and severity, although minimal to slight, of thyroid follicular cell hypertrophy in the female rats, as well as in the male rats. Rats do not produce glucuronidated silodosin, which is present in human serum at approximately 4 times the level of circulating silodosin and which has similar pharmacological activity and distribution in animal studies.

Relevance to humans of tumors observed in carcinogenicity studies:

The Pharmacology/Toxicology Carcinogenicity Assessment Committee concluded that the thyroid follicular cell adenomas in male rats were drug related. Although the incidence of follicular cell adenomas in female rats was increased, the incidences in dosed groups were not statistically significant and thus not clearly related to the drug. However, the committee noted that the increased incidence and severity, although minimal to slight, of thyroid follicular cell hypertrophy in the female rats, as well as in the male rats, suggests that the thyroid of females is also a potential organ for toxicity of the drug. The committee concluded that the mammary gland adenoacanthomas in female mice were drug related. The committee also concluded that the mammary gland adenocarcinomas, and adenomas or carcinomas, were drug related.

Evidence of a mechanism in rats exist that may not be relevant to humans: In rats, drug-induced thyroid tumors are sometimes reported to be induced by increased UDP-GT levels and resulting alterations of thyroid hormones. Studies using silodosin were performed and confirmed the presence of this mechanism in rats after silodosin administration. No evidence of an effect of silodosin on thyroid hormones or on prolactin levels was observed in adult male clinical trial participants.

In mice, a study was conducted which determined that levels of prolactin increased after silodosin administration. Mechanistically, the tumors (mammary and non-statistically significant pituitary tumors) were attributed to increased production and secretion of prolactin in the pituitary, caused by an inhibition of dopamine in the hypothalamus. Clinically, the induction of these tumors in mice is not usually considered relevant, because the drug is not indicated in females, there is a sufficient safety margin between the doses at which the tumors were noted and the clinical dose, and because induction of mammary adenomas and carcinomas have been noted in mice after administration of other drugs of this class without clinical findings in adult male humans.

The sponsor also provided documentation of a similar prolactin related mechanism associated with mammary adenoacanthomas, as well as adenocarcinomas, in female mice (see Abilify label).

An embryo/fetal study in rabbits showed decreased maternal body weight at the high dose of 200 mg/kg/day (approximately 13-25 times the maximum recommended human exposure of parent drug via AUC). No evidence of teratogenicity was observed at this dose. Variations of lung lobation were observed at 20, 60, and 200 mg/kg/day and one fetus in each treated group (< 1%, not statistically significant) had a ventricular septal defect.

Embryo/fetal studies in rats showed no maternal or fetal effects at a high dose of 1000 mg/kg/day.

In a combined male/female rat fertility study, at 60 mg/kg/day and above, prolongation or disappearance of the estrous cycle was observed in females. Decreased copulation index was observed at 200 mg/kg/day and above and decreased fertility index was observed at 20 mg/kg/day and above (all treated doses).

In a male rat fertility study, sperm viability and count were significantly lower in the 600 mg/kg/day (about 65 times the exposure of the maximum recommended human dose via AUC) group after one month. Histopathological examination of infertile males revealed changes in the testes and epididymides in the 200 (about 30 times) and 600 mg/kg groups which were considered to be due to treatment with KMD-3213. The copulation and fertility indices indicated no significant differences between the treated groups and the control group. However, the fertility index was somewhat lower in the 600 mg/kg group. Implantation index observed at cesarean section was significantly lower in the 600 mg/kg group. The no-observed adverse effect level (NOAEL) of KMD-3213 for general toxicity was 200 mg/kg in male rats. The NOAEL of KMD-3213 for male reproductive function was 60 mg/kg and that for early embryonic development was 200 mg/kg. These effects are at relatively high multiples of expected clinical exposures.

Treatment of male rats with silodosin for 15 days resulted in decreased fertility and implantation index at the high dose of 20 mg/kg/day (about twice the exposure of the maximum recommended human dose via AUC). Effects on fertility and implantation

indices recovered after a 2 weeks recovery period. No effect was observed at 6 mg/kg/day. The high dose effects appear to be in an exposure range which may be relevant to clinical use, similar to effects reported for other drugs in this class.

In a female rat fertility study, no effect on fertility parameters was observed at the high dose of 20 mg/kg/day (about 1 to 4 times the exposure of the maximum recommended human dose via AUC). This dose did result in estrus cycle changes. No effect on the estrus cycle was observed at 6 mg/kg/day. Silodosin is not approved for use in women.

No effects on physical or behavioral development of offspring were observed when rats were treated during pregnancy and lactation at up to 300 mg/kg/day.

In an evaluation of silodosin *in vitro* for phototoxicity in Balb/c 3T3 fibroblasts using a neutral red assay, a small increase in phototoxicity over control (classified as a "probable" level of phototoxicity) was observed in the presence of silodosin. However, in a single dose oral phototoxicity study in hairless mice, only mild erythema was observed after 4 hours simulated sunlight exposure at high silodosin exposure levels.

2.6.6.10 Tables and Figures

Included in text.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) [a]	GLP Compliance	Testing Facility	Study Number
Single-Dose Toxicity	Rats, Slc:SD	Forced oral	–	500, 1000, 2000 [c]	Applicable	Kissei [d]	00229
		Forced oral	–	400, 800, 1600	Non-applicable	Kissei [d]	10017
		Intravenous	–	0, 60, 75, 90	Applicable	Kissei [d]	10092
	Dogs, beagle	Capsule	–	1500, 2000 [b]	Applicable	Kissei [d]	00233
		Capsule	–	1000, 1500	Applicable	Kissei [d]	10025
		Intravenous	–	25, 50	Applicable	Kissei [d]	10093
Repeat Dose Toxicity	Rats, [redacted]:SD	Forced oral	28 days	0, 50, 150, 300	Applicable	[redacted]	KMD-3213-IT-PH-0233
	Rats, Slc:SD	Forced oral	1 month	0, 30, 100, 300, 800	Non-applicable	Kissei [d]	00238
		Forced oral	1 month	0, 20, 60, 200, 600	Applicable	Kissei [d]	10026
		Forced oral	3 months	0, 25 [e], 100 [d], 400	Applicable	Kissei [d]	10077
		Forced oral	26 weeks	0, 15, 60 [d], 300	Applicable	Kissei [d]	10081
		Forced oral	26 weeks	0, 1, 5	Applicable	Kissei [d]	10111
		Intravenous	2 weeks	0, 2, 10, 50	Applicable	Kissei [d]	10242
	Dogs, beagle	Capsules	2 weeks	50, 200, 500	Non-applicable	Kissei [d]	70158
		Capsules	1 month	0, 25, 100, 400	Applicable	Kissei [d]	10008
		Capsules	13 weeks	0, 10, 50, 100/200 [e]	Applicable	[redacted]	KSI 70/970908
		Capsules	52 weeks	0, 5, 20, 80	Applicable	[redacted]	KSI 71/974423
		Intravenous	2 weeks	0, 1, 5, 25	Applicable	Kissei [d]	10236

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Genotoxicity	*Salmonella typhimurium* and *Escherichia coli*	*in vitro*	–	0, 46.9, 93.8, 187.5, 375, 750, 1500, 3000 µg/plate	Applicable	Kissei [d]	10036
	Mouse lymphoma cells (L5178Y)	*in vitro*	–	Test without metabolic activation 1: 0, 60, 125, 250, 375 µg/mL Test with metabolic activation 1: 0, 60, 125, 200, 300 µg/mL Test without metabolic activation 2: 0, 150, 200, 250, 300 µg/mL Test with metabolic activation 2: 0, 200, 250, 300, 350 µg/mL	Applicable	[redacted]	KSI 80/973223
	Chinese hamster lung cells (CHL)	*in vitro*	–	Continued treatment without metabolic activation: 0, 21.9, 43.8, 87.5 µg/mL Short-term treatment method with and without metabolic activation: 0, 87.5, 175, 350 µg/mL Short-term treatment method without metabolic activation (confirmatory test): 0, 50.0, 200, 350, 500 µg/mL	Applicable	[redacted]	2626 (005-013)

b(4)

b(4)

b(4)

b(4)

b(4)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Genotoxicity (cont)	Chinese hamster lung cells (CHL/IU)	*in vitro*	–	Short-term treatment method without metabolic activation: 0, 37.5, 75, 150, 300, 600 μg/mL	Applicable	[redacted]	7L425
	Mice, [redacted]CD-1(ICR)	Forced oral	Preliminary toxicity study 1, 2: single Preliminary test single or twice	Preliminary toxicity study 1: 10, 100, 1000, 2000 Preliminary toxicity study 2: 1600, 2900, 5000 Preliminary micronuclens test: 1000	Non-applicable	Kissei [a]	50048
	Mice, [redacted]CD-1(ICR)	Forced oral	Single	0, 250, 500, 1000	Applicable	Kissei [a]	10067
	Rats, Hsd/Ola:SD	Forced oral	Single	0, 600, 2000	Applicable	[redacted]	KSI 083/974372

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Carcinogenicity	Mice, [redacted]CD-1(ICR)	Mixed with food	14 days	0, 400, 600, 800	Applicable	[redacted]	KSI 089/982293
	Mice, [redacted]CD-1(ICR)	Mixed with food	13 weeks	0, 200, 400, 800	Applicable (Non-applicable [b])	[redacted]	KSI 086/982491, KSI 098/980210, KSI 111/990049 [b]
	Mice, [redacted]CD-1(ICR)	Mixed with food	102 weeks	Female: 0, 0, 60, 150, 400	Applicable	[redacted]	KSI 100/012988
	Mice, [redacted]CD-1(ICR)	Mixed with food	104 weeks	Male: 0, 0, 20, 60, 100/200 [a]	Applicable	[redacted]	KSI 114/012990
	Rats, [redacted]CD(SD)	Mixed with food	14 days	0, 400, 600, 800	Applicable	[redacted]	KSI 088/982292
	Rats, [redacted]CD(SD)	Mixed with food	13 weeks	0, 30, 125, 500	Applicable (Non-applicable [b])	[redacted]	KSI 084/982477, KSI 099/980211, KSI 112/990048 [b]
	Rats, [redacted]CD(SD)	Mixed with food	104 weeks	Male: 0, 0, 15, 50, 150 Female: 0, 0, 15, 80, 250	Applicable	[redacted]	KSI 102/012989

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Reproductive and Developmental Toxicity	Rats, [redacted]CD(SD)	Forced oral	[a]	0, 20, 60, 200, 600	Applicable	Kissei [a]	10006
	Rats, [redacted]CD(SD)	Forced oral	[b]	0, 20, 60, 200, 600	Applicable	Kissei [a]	10059
	Rats, [redacted]CD(SD)	Forced oral	[c]	0, 2, 6, 20	Applicable	Kissei [a]	10112
	Rats, [redacted]CD(SD)	Forced oral	[d]	0, 0.6, 2, 6, 20	Applicable	Kissei [a]	10072
	Rats, [redacted]CD(SD)	Forced oral	F: G7 - G17 [e]	0, 200, 400, 600	Non-applicable	Kissei [a]	50033
	Rats, [redacted]CD(SD)	Forced oral	F: G7 - G17 [e]	0, 30, 80, 240, 700	Applicable	Kissei [a]	10058
	Rats, [redacted]CD(SD)	Forced oral	F: G7 - G17 [e]	0, 1000	Applicable	Kissei [a]	10140
	Rabbits, Kbl:NZW	Forced oral	F: G6 - G18 [e]	0, 50, 100, 200, 400, 600	Non-applicable	Kissei [a]	50034
	Rabbits, Kbl:NZW	Forced oral	F: G6 - G18 [e]	0, 20, 60, 200	Applicable	Kissei [a]	10050, 10116 [c]
	Rats, [redacted]CD(SD)	Forced oral	F: G7 - L20 [e]	0, 30, 100, 300, 600	Non-applicable	Kissei [a]	50075
	Rats, [redacted]CD(SD)	Forced oral	F: G7 - L20 [e]	0, 10, 30, 100, 300	Applicable	Kissei [a]	10101

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Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Local Tolerance	Human blood	*in vitro*	–	silodosin for injection for clinical pharmacological study (0.2 mg/mL)	Applicable	[redacted]	2002IF006
	Rabbits, Kbl:NZW	Intramuscular	Single dose	silodosin for injection for clinical pharmacological study (0.2 mg/mL)	Applicable	[redacted]	2002IF005
Other Toxicity Studies							
Antigenicity studies	Mice, BALB/c Cr Slc and Mice, C3H/He Slc	Intraperitoneal	Once a week for 2 weeks	silodosin: 10, 100 µg/animal silodosin conjugated with mouse serum albumin: Protein eq. 10 µg/animal	Applicable	Kissei [b)]	10037
	Guinea pigs Slc:Hartley	Subcutaneous	Once a week for 3 weeks	silodosin: 0.025, 0.075 mg/animal silodosin conjugated with guinea pig serum albumin: Protein eq. 1 mg/animal	Applicable	Kissei [b)]	10069

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Other Toxicity Studies (continued)							
Phototoxicity Study	Balb/c 3T3 fibroblast cells	*in vitro*	Prior to UV-A exposure: 44 hrs to 44 hrs, 43 min +/- UV-A exposure: 81 min, 57 sec to 109 min, 2 sec After UV-A exposure: 18 hrs, 30 min to 19 hrs, 10 min	0.316 to 1000 µg/mL	Applicable	[redacted]	KMD-3213-IT-PH-0236
Studies of protease inhibitory effect	Trypsin (derived from bovine pancreas)	*in vitro*	–	0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 µg/mL	Applicable	Kissei [b)]	10281
	Papain (derived from papaya latex)	*in vitro*	–	0, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10 µg/mL	Applicable	Kissei [b)]	10282

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Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Studies on metabolites	Mice, Slc:ICR	Forced oral	Single dose	Male: 20, 100, 500 Female: 60, 150, 400	Applicable	Kissei [a)]	10235
	Rats, Slc:SD	Forced oral	Single dose	100, 300, 600	Applicable	Kissei [a)]	10216
	Dogs, beagle	Forced oral	Single dose	100, 200	Applicable	Kissei [a)]	10221
Studies on blood hormones	Mice, Slc:ICR	Forced oral	Single dose, 2 weeks	0, 6, 20, 60, 200	Applicable	Kissei [a)]	10220
	Rats, Slc:SD	Forced oral	Single dose, 2 weeks	0, 5, 15, 50, 150	Applicable	Kissei [a)]	10219
Study on hypertrophy of the thyroid	Rats, Slc:SD	Forced oral	4 weeks	0, 150, 300	Applicable	Kissei [a)]	10283
Study on accumulation of metabolites in the liver and kidney	Dogs, beagle	Capsules	4 weeks	25	Applicable	[redacted]	KSI 115/994798

b(4)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	GLP Compliance	Testing Facility	Study Number
Toxicity studies of the glucuronide conjugate metabolite of silodosin	Rats, Slc:SD	Intravenous	Single	10, 50, 100 mg/kg	Applicable	Kissei [a)]	10299
	Rats, Slc:SD	Intravenous	Single	10, 50 mg/kg	Applicable	Kissei [a)]	10301
(KMD-3213G or MD127K)	Rats, Slc:SD	Intravenous	Single	(0.1 mol/L citric acid buffer) 5, 7.5, 10 mL/kg	Non-applicable	Kissei [a)]	50345
	Rats, Slc:SD	Intravenous	2 weeks	0, 2, 10, 50 mg/kg	Applicable	Kissei [a)]	10302, 10308 [b)]
	Salmonella typhimurium and *Escherichia coli*	*in vitro*	–	0, 78, 156, 313, 625, 1250, 2500, 5000 µg/plate	Applicable	Kissei [a)]	10292
	Chinese hamster lung cells (CHL/IU)	*in vitro*	–	0, 1250, 2500, 5000 µg/mL	Applicable	Kissei [a)]	10298

Type of Study	Test System	Method of Administration	Doses (mg/kg)	GLP Compliance	Study No.
Single dose study	Rats	Intravenous	60, 75, 90	Applicable	10092
Single dose study	Dogs	Intravenous	25, 50	Applicable	10093
1 month dose study	Rats	Forced oral	0, 20, 60, 200, 600	Applicable	10026
26-week dose study (1)	Rats	Forced oral	0, 15, 60, 300	Applicable	10081
26-week dose study (2)	Rats	Forced oral	1, 5	Applicable	10111
2-week dose study	Rats	Intravenous	2, 10, 50	Applicable	10242
1 month dose study	Dogs	Capsules	25, 100, 400	Applicable	10008
13-week dose study	Dogs	Capsules	10, 50, 100/200 [a)]	Applicable	KSI 70/970908
52-week dose study	Dogs	Capsules	5, 20, 80	Applicable	KSI 71/974423
2-week dose study	Dogs	Intravenous	1, 5, 25	Applicable	10236
13-week dose-finding study	Mice	Mixed with food	200, 400, 800	Applicable	KSI 086/982491
Carcinogenicity study	Female mice	Mixed with food	60, 150, 400	Applicable	KSI 100/012988
Carcinogenicity study	Male mice	Mixed with food	20, 60, 100/200 [b)]	Applicable	KSI 114/012990
13-week dose-finding study	Rats	Mixed with food	30, 125, 500	Applicable	KSI 084/982477
Carcinogenicity study	Rats	Mixed with food	Male: 15, 50, 150 Female: 15, 80, 350	Applicable	KSI 102/012989
Toxicokinetic study	Rabbits	Forced oral	20, 60, 200	Applicable	10116
Study on metabolites	Mice	Forced oral	20, 60, 100, 150, 400, 500 [c)]	Applicable	10235
Study on metabolites	Rats	Forced oral	100, 300, 600	Applicable	10216
Study on metabolites	Dogs	Capsules	100, 200	Applicable	10221
4-week study of accumulation of metabolites in the liver and kidney	Dogs	Capsules	25	Applicable	KSI 115/994798

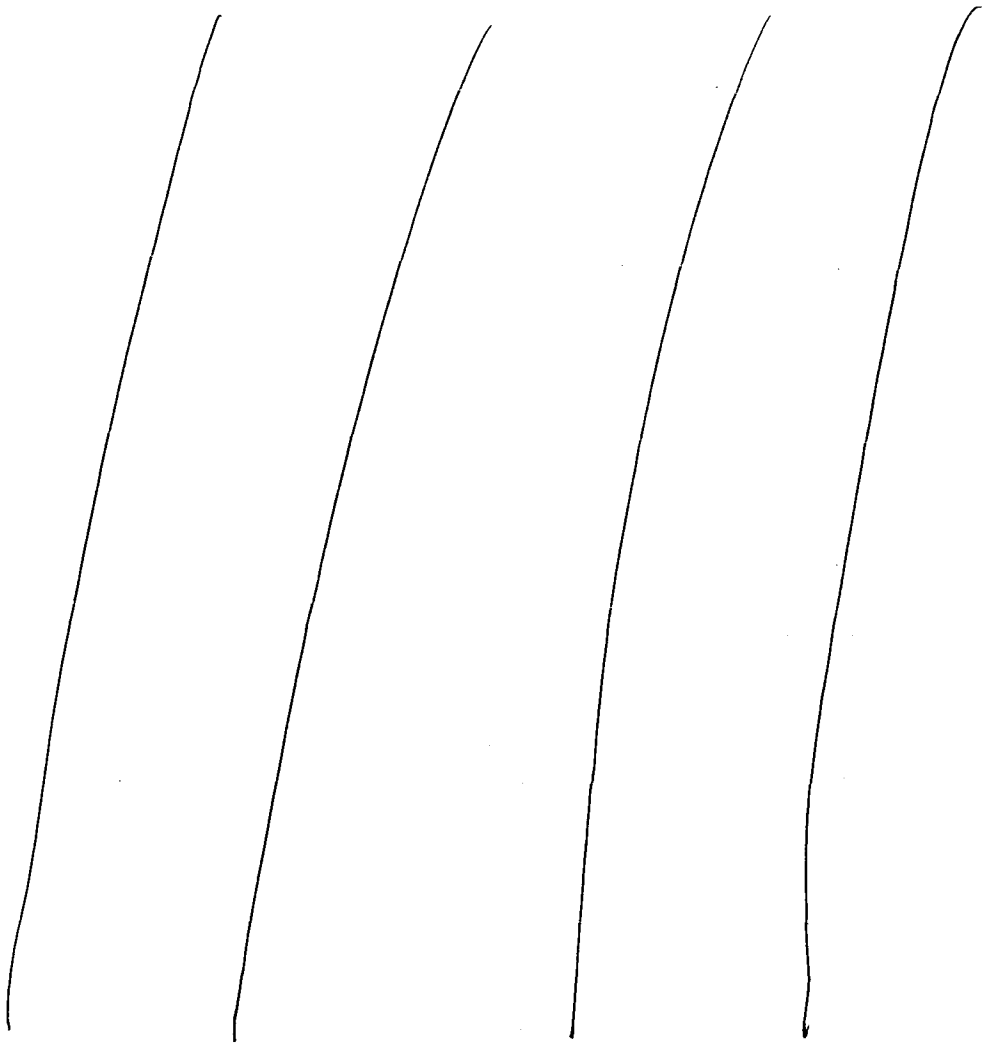
a) The investigational article was administered initially at 200 mg/kg, which was decreased to 100 mg/kg on Day 7.
b) The investigational article was administered initially at 200 mg/kg, which was decreased to 100 mg/kg at Week 27.
c) Male: 20, 100 and 500 mg/kg, Female: 60, 150 and 400 mg/kg.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions/Recommendations: There is no impediment to approval from a Pharmacology/ Toxicology perspective

Unresolved toxicology issues (if any): none

Suggested labeling: See Executive Summary for clean copy.



1 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

_____ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Laurie McLeod
9/9/2008 12:00:52 PM
PHARMACOLOGIST

Lynnda Reid
9/11/2008 08:35:28 AM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

NDA Number: 22206 **Applicant: Watson Laboratories, Inc.** **Stamp Date: 12/13/07**

Drug Name: Silodosin **NDA/BLA Type: original**

On **initial** overview of the NDA/BLA application for RTF:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		52-week dog and 26-rat studies, rat and mouse 2-year carcinogenicity bioassays, rat and rabbit embryo-fetal assays, rat fertility and development assays, genotoxicity, comparative toxicokinetics, ADME, safety pharmacology and pharmacology studies are completed.
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Genotoxicity and 2-week intravenous studies of the glucuronide metabolite, not produced by the toxicity species, are complete.

File name: 5_Pharmacology_Toxicology Filing Checklist for a New NDA [redacted]

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?		X	b(4)
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	Has the applicant addressed any abuse potential issues in the submission?		X	No dependence studies were conducted; however, dependence or other abuse potential is not expected for this class of drug.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			NA

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? __yes____

List of submitted studies:
28-Day Oral Dose Study with Interim Sacrifices and 30-Day Recovery in Rats
One-Month Oral Dose Study and One-Month Recovery Study in Rats
Three-Month Oral Dose Study in Rats
Twenty-Six-Week Oral Dose Study in Rats (First Study)
Twenty-Six-Week Oral Dose Study in Rats (Second Study)
Two-Week Intravenous Injection Study in Rats
One-Month Oral Dose Study and One-Month Recovery Study in Dogs
Thirteen-Week Oral Dose Study in Dogs
Fifty-Two-Week Oral Dose Study in Dogs
Two-Week Intravenous Injection Study in Dogs
In Vitro Reverse Mutation Test with Bacteria
Mouse Lymphoma Assay
Chromosomal Aberration Test with Mammalian Cells in Culture (First Study)
Chromosomal Aberration Test with Mammalian Cells in Culture (Second Study)
In Vivo Micronucleus Test with Mice
Unscheduled DNA Synthesis (UDS) Test with Rat Hepatocytes
Fourteen-Day Palatability Study in Mice
Thirteen-Week Dose Finding Study in Mice
One-Hundred-Four-Week Carcinogenicity Study in Female Mice
One-Hundred-Four-Week Carcinogenicity Study in Male Mice
Fourteen-Day Palatability Study in Rats
Thirteen-Week Dose-Finding Study in Rats
One-Hundred-Four-Week Carcinogenicity Study in Rats
Reproductive and Developmental Toxicity - Fertility and Early Embryonic Development to Implantation (Pivotal)
Study on Fertility and Early Embryo Development Until Implantation in Rats

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

Study on Fertility and Early Embryo Development Until Implantation in Rats - Study of Dose in Male Rats (First Study)
Study on Fertility and Early Embryo Development Until Implantation in Rats - Study of Dose in Male Rats (Second Study)
Study on Fertility and Early Embryo Development Until Implantation in Rats - Study of Dose in Female Rats
Reproductive and Developmental Toxicity - Effects on Embryo-Fetal Development (Pivotal)
Embryo-Fetal Development Study in Rats (First Study)
Embryo-Fetal Development Study in Rats (Second Study)
Embryo-Fetal Development Study in Rabbits
Reproductive and Developmental Toxicity - Effects on Pre- and Postnatal Development, Including Maternal Function (Pivotal)
Antigenicity
Phototoxicity
In Vitro Study of Inhibitory Effect on Trypsin
In Vitro Study of Inhibitory Effect on Papain
Study of Metabolites after a Single Oral Dose in Mice
Study of Metabolites after a Single Oral Dose in Rats
Study of Metabolites after a Single Oral Dose in Dogs
Study on Blood Hormone Levels after a Oral Dose in Mice
Study on Blood Hormone Levels after a Oral Dose in Rats
Study on Hypertrophy of the Thyroid by Repeated Oral Dose in Rats
Four-Week Oral Dose Study to Investigate Accumulation of Metabolites in the Liver and Kidney in Dogs
Single Intravenous Injection Study of Glucuronide Conjugate of Silodosin in Rats
Two-Week Intravenous Injection Study of the Glucuronide Conjugate of Silodosin in Rats
Reverse Mutation Test of the Glucuronide Conjugate of Silodosin in Bacteria
Chromosomal Aberration Test of the Glucuronide Conjugate of Silodosin with Mammalian Cells in Culture

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

In the animal toxicology sections of the label, doses are expressed as nominal doses. Comparative exposures should also be expressed in terms of multiples of AUC of the maximum recommended clinical exposures for both reproductive and carcinogenicity studies. Multiples should be expressed as multiples of parent drug and as multiples of parent drug plus the major human glucuronide metabolite since the secondary glucuronide metabolite was not tested as a separate entity.

A "probable" level of phototoxicity (2- to 4-fold) was observed in an *in vitro* study. An *in vivo* phototoxicity study and *in vitro* photo-genotoxicity studies should be performed to determine if a clinical phototoxicity study is indicated. b(4)

Please provide documentation that supports the theoretical link between mammary adenoacanthomas and prolactin levels in CD mice as being similar to the reported link between prolactin levels and adenocarcinoma. Interpretation of the results and labeling b(4) continue to be under review.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR A NEW NDA/BLA

Reviewing Pharmacologist Date

Team Leader/Supervisor Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Laurie McLeod
1/29/2008 10:07:55 AM
PHARMACOLOGIST

Lynnda Reid
1/30/2008 08:18:33 AM
PHARMACOLOGIST