CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

NDA 22-206

PHARMACOLOGY REVIEW(S)

MEMO **FOOD AND DRUG ADMINISTRATION**

Division of Reproductive and Urologic Products
Center for Drug Evaluation and Research

Date:	September 12, 2008
Reviewer:	Lynnda Reid, Supervisory Pharmacologist, DRUP
NDA #/SS#/date:	22-206/N000/December 13, 2007
Sponsor:	Watson laboratories, Inc.
Drug Product:	Rapaflo® (Silodosin)
Indication:	Benign Prostatic Hyperplasia (BPH)
Recommended Action:	Nonclinical data support approval.

Background: Silodosin was investigated as a treatment ———————— signs and symptoms associated with benign prostatic hyperplasia (BPH) under IND 56,605. Nonclinical studies included in vitro receptor binding studies, pharmacology and safety pharmacology studies, acute, subchronic and chronic toxicology studies in rats and dogs, in vitro and in vivo genotoxicity assays, carcinogenicity 2-year bioassays in rodents, and reproductive and developmental toxicity in rats and rabbits. b(4)

Pharmacology:
Primary Mechanism of Action: Silodosin is an apha-1 adronoceptor antagonist. In nonclinical models, silodosin blocks the sympathetic nervous system via the α_{1A}-AR subtype distributed in the prostate, urethra and trigone of the bladder to relieve tension of the smooth muscles of the lower urinary tract tissues. The reaction results in persistent decreases in urethral pressure, thereby improving overactive bladder-like symptoms observed in the hormone induced BPH rat model.

Secondary Pharmacology: Silodosin also binds to the α_{1B}-AR, α_{1D}-AR, α_2-AR, β_1-AR, β_2-AR, muscarinic receptor, serotonin (5-HT1) receptor and dopamine (D1, D2 long, D3 and D4.2) receptors with significantly less affinity than to α_{1A}-AR.

Safety Pharmacology: Neurological effects (trembling, somnolence and decreased body temperature) were observed in rats at 20 mg/kg, but not in dogs following oral administration. In dogs, decreased blood pressure was noted at all oral doses (0.2 – 20 mg/kg) following oral administration but not following iv doses (0.3 – 3 mg/kg). No effect was seen on QT interval in vivo, although in vitro, silodosin inhibited the hERG with IC_{25} and IC_{50} values of 3.72 and 8.91, respectively.

Metabolism: The main metabolite in rats and dogs was KMD-3310, while the glucuronide conjugate of silodosin KMD-3123G (aka MD127K) and KMD-3293 were the major human metabolites. KMD-3123G is not found in any of the animal models (mouse, rat, rabbit and dog) used in the nonclinical program. The affinities of KMD-3123G and KMD-3293 for α_{1A}-AR 8- and 24-fold lower than silodosin, while the major animal metabolite KMD-3310 had no affinity for α_{1A}-AR. In isolated rat prostate tissue, both silodosin and KMD 3213G caused the concentration-response curve of noradrenaline-induced contractions to decrease.

Toxicology: Potential adverse effects following chronic administration were assessed in rats (6-months) and dogs (1 year) utilizing oral administration. In addition, a 2-week intravascular bridging study was conducted in rats with the active glucuronide conjugate, KMD-3213G (aka MD127K) which is only produced in humans.

In rats and dogs, the most common findings were clinical signs associated with exaggerated pharmacologic effects, e.g., lacrimation, salivation and ptosis. Non dose-limiting effects were observed in the livers of both rats and dogs, including slight to moderate fatty degeneration of hepatocytes in rats and lipid accumulation in dogs. In rats, silodosin administration was also associated with slight swelling and eosinophilic changes in centrilobular hepatocytes in males and females, slight dilation of the adrenal cortex in males, and hypertrophy of the vaginal mucous epithelium and slight mammary gland hyperplasia in females.

In vitro and in vivo phototoxicity studies suggest that silodosin may have the ability to minimally increase sensitivity to sunlight.

The toxicity profile for KMD-3123G following 2-week i.v. administration in rats was comparable to the pharmacologic and toxicologic effects observed with i.v. silodosin.

Genotoxicity: The weight of evidence suggests that silodosin is not genotoxic. Silodosin was negative in the Ames Assay, in the mouse lymphoma cell assay, mouse micronucleus assay at doses up to 1000 mg/kg, and in a rat liver DNA repair (UDS) assay. In the chromosomal aberration assay, silodosin was negative at 6, 24 and 48 hours with metabolic activation and at 24 and 48 hours without metabolic activation. However, there was a slight increase in the number of chromosomal aberrations at 6 hours in the absence of metabolic activation. Further analysis demonstrated that there was also a decreased in the mitotic index, indicative of cellular toxicity.

KMD-3123G was negative in the Ames Assay and chromosomal aberration assay.

Carcinogenicity: Carcinogenicity risk associated with chronic administration of silodosin was evaluated in 2-year studies in male and female rats and mice. In female mice there was an increase in mammary gland adeneoacanthomas and adenocarcinomas. These findings appear to be related to increased prolactin production in mice

In male rats, there was an increased incidence of thyroid follicular cell tumors. These tumors were related to increased UDP-GT levels, a common finding specific to rodents.

Alternations in prolactin or thyroid hormone levels were assessed in clinical trial subjects, with no reported changes. Therefore any potential for increased carcinogenicity risk associated with chronic exposures to silodosin is considered minimal.

The potential for KMD-3123G to increase carcinogenicity risk was not evaluated.

Reproductive and developmental toxicology: Potential reproductive and developmental toxicity was evaluated in rats and rabbits. There was no evidence of teratogenicity in rats or rabbits which support a Pregnancy Category B. Slight, but reversible, effects in male rats consisted of decreases in sperm counts and mobility. In female rats, silodosin was associated with alterations in estrus cycles at high doses, but not with infertility.

In dogs, maturation of testes and epididymis and absence of sperm was observed at doses approximately 5 times higher than the maximum recommended human dose at 13 weeks, but not after 52 weeks of dosing.

The potential for KMD-3123G to induce reproductive and developmental toxicity was not evaluated.

Outstanding nonclinical issues: None.

b(4)

Labeling: I concur with the labeling proposed by Dr. McLeod-Flynn with the exception of changing the wording, as suggested by Dr. Jacobs, to reflect that the relevance to humans of the thyroid tumors observed in rats is unknown.

Conclusion: I concur with the primary nonclinical reviewer, Dr. Laurie McLeod-Flynn, that the nonclinical data support an approval action for this NDA.

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/s/

Lynnda Reid
9/15/2008 09:57:57 AM
PHARMACOLOGIST

Comments on NDA 22-206 Silodosin
From A. Jacobs 9/11/08

I concur that there are no outstanding pharm/tox issues.

b(4)

I concur with the proposed pregnancy category: B

In the labeling under carcinogenesis,

Suggested rewording: relevance to human risk of these thyroid tumors in rats is not known

I have discussed this with the pharm/tox reviewer and supervisor.

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

/s/

Abby Jacobs
9/11/2008 07:43:58 AM
PHARMACOLOGIST

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-206**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**12/13/07**
PRODUCT:	**Silodosin**
INTENDED CLINICAL POPULATION:	**men, for the treatment of signs and symptoms of benign prostatic hyperplasia (BPH)**
SPONSOR:	**Watson Laboratories, Inc.**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Reproductive and Urologic Products (HFD-580)**
PHARM/TOX REVIEWER:	**Laurie McLeod-Flynn, Ph.D., D.A.B.T.**
PHARM/TOX SUPERVISOR:	**Lynnda Reid, Ph.D.**
DIVISION DIRECTOR:	**Scott Monroe. M.D.**
PROJECT MANAGER:	**Olga Salis**

Date of review submission to Division File System (DFS): 09 September, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: There is no impediment to approval of this drug from a Pharmacology/Toxicology perspective.

B. Recommendation for nonclinical studies: No recommendation for further nonclinical studies is being made at this time.

C. Recommendations on labeling

8 USE IN SPECIFIC POPULATIONS

8.1 PREGNANCY

b(4)

13 NONCLINICAL TOXICOLOGY

13.1 CARCINOGENESIS, MUTAGENESIS, AND IMPAIRMENT OF FERTILITY

b(4)

b(4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings:

Silodosin was studied in dogs (up to 52 weeks, up to about 51 – 118 times the expected clinical exposure via AUC) and in rats (up to 26 weeks, up to about 20 times the clinical AUC). Brown discoloration, enzyme induction/hepatocellular hypertrophy, and lipid droplets were observed in liver of both species at all doses. No indication of hepatic toxicity was observed in dogs, but fatty degeneration of hepatocytes was observed in rats at all doses tested (and in the control group), with some dose dependence.

The major human glucuronidated metabolite of silodosin, MD127K, was found to be similar both in pharmacology and toxicology to the parent drug. Pharmacology studies showed this metabolite, which circulates in humans at about 4 times the concentration of

silodosin, to be slightly less active than the parent drug, and distribution studies in rats showed it to be distributed to tissue, including the prostate. This metabolite is not produced by rats, dogs, or mice. If silodosin and its active glucuronidated metabolite concentrations were considered additive, activities and toxicities dependent on this metabolite might be considered to occur at lower multiples in animals studies compared to human studies than reported (possibly by two to three times); however, conclusions as to approvability of silodosin from a pharmacology/toxicology perspective would not change. All other human metabolites were present in animal studies.

Silodosin showed no evidence of mutagenic or genotoxic potential in the *in vitro* Ames assay, mouse lymphoma assay, unscheduled DNA synthesis assay and the *in vivo* mouse micronucleus assay. A weakly positive response was obtained in two *in vitro* Chinese Hamster Lung (CHL) test for chromosomal aberration assays at high, cytotoxic concentrations. MD127K showed no evidence of genotoxic potential.

In a 2-year carcinogenicity study in mice at about 19 times the exposure of the maximum recommended human dose or MHRE via AUC in males and 68 times the MRHE via AUC in females, there were no significant tumor findings in male mice. Female mice treated for 2 years with about 29 times the MRHE or greater had statistically significant increases in the incidence of mammary gland adenoacanthoma and adenocarcinomas. In a 2-year study in rats administered doses up to about 8 times the exposure of the maximum recommended human dose or MRHE via AUC of silodosin, an increase in thyroid follicular cell tumor incidence was seen in male rats.

An embryo/fetal study in rabbits showed decreased maternal body weight at approximately 13-25 times the maximum recommended human exposure of parent drug via AUC). No evidence of teratogenicity was observed at this dose.

Embryo/fetal studies in rats showed no maternal or fetal effects at approximately 20 times the MRHE.

In male rats, fertility was decreased at about 2 times the MRHE. No effect on fertility was observed after a 2 week recovery period. Sperm viability and count, and changes in the testes and epididymus, were observed at very high doses in rats. In dogs a NoAEL for delayed maturation of testes and epididymus, and absence of sperm, was 10 mg/kg/day.

In a female rat fertility study, no effect on fertility parameters was observed at about 1 – 4 times the MRHE; however, effects on the estrus cycle were observed.

Phototoxicity of silodosin was observed *in vitro* and *in vivo*.

B. Pharmacologic activity: Silodosin is an alpha-1 adrenoceptor antagonist.

C. Nonclinical safety issues relevant to clinical use:

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 reported to be induced by increased UDP-GT levels and resulting alterations of thyroid hormones. This mechanism was confirmed by direct measurement of thyroid hormones 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 the documented relationship in rodents between 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 another drug with a similar prolactin related mechanism associated with mammary adenoacanthomas, as well as adenocarcinomas, in female mice (see Abilify label). Mammary adenoacanthomas are also sometimes associated with age in rodents.

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. Brown discoloration of the liver at low doses is sometimes considered due to lipofuscin formation, which is sometimes correlated with inhibition of proteolysis, oxidative stress, and with aging. No indication of proteolysis inhibition was observed in *in vitro* studies. Oxidative stress was not studied. It is not known what relevance that these effects, often seen in animals, may have clinically. No clear signal of liver toxicity was observed in clinical trials. Clinical monitoring of liver effects will continue into phase IV of development.

Evidence of phototoxicity was primarily observed at high doses in animals and *in vitro*. No drug related phototoxic or skin effects were observed in the clinic.

Chromosomal aberrations in cell culture at high, cytotoxic doses, are not expected to be relevant to clinical use, as they are considered secondary to severe cytotoxicity.

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. This effect appears 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 male rat fertility study, 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). These effects are at relatively high multiples of expected clinical exposures.

In a fertility study in female rats, the high dose of 20 mg/kg/day (about 1 – 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. Silodosin is not approved for use in women.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

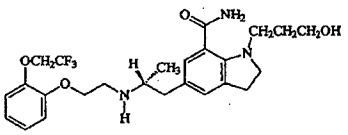
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22206
Review number: 1
Sequence number/date/type of submission: 000/13 December 2007/original submission
Information to sponsor: Yes ( ) No (x)
Sponsor and/or agent: Watson Laboratories, Inc.
Manufacturer for drug substance: Watson Laboratories, Inc.

Reviewer name: Laurie McLeod-Flynn
Division name: Division of Reproductive and Urologic Products
HFD #: 580
Review completion date: 13 August 2008

Drug:

Trade name: Rapaflo
Generic name: Silodosin
Code name: KMD-3213
Chemical name: (-)-(R)-1-(3-hydroxypropyl)-5-[2-[[2-[2-(2,2,2-trifluoroethoxy) phenoxy]-ethyl] amino] propyl] indoline-7-carboxyamide
Molecular formula/molecular weight: $C_{25}H_{32}F_3N_3O_4$ / 495.54
Structure:



Relevant INDs/NDAs/DMFs: DMF —— , IND 56605 b(4)

Drug class: alpha-1 adrenoceptor antagonist

Intended clinical population: men, for the treatment of signs and symptoms of benign prostatic hyperplasia (BPH)

Clinical formulation: 4- and 8- mg tablets

Route of administration: oral

Studies reviewed within this submission:

Pharmacology:

Receptor Binding Study of KMD-3213 and Its Metabolites or Optical Isomer (KMD-0005-A)
Effects of KMD-3213 on Blood Pressure, Heart Rate, and Phenylephrine-Induced Increase of Intraurethral Pressure in Rats: Intravenous Administration (KMD-0006-A)
Effects of KMD-3213 on Noradrenaline-Induced Contractions of Isolated Thoracic Aorta and Spleen in Rats (KMD-0007-A)
Effects of KMD-3213 on Blood Pressure, Heart Rate, and Phenylephrine-Induced Increase of Intraurethral Pressure in Rats: Intraduodenal Administration (K<D-0013-A)
Effects of KMD-3213 on Hypogastric Nerve Stimulation-Induced Increase of Intraurethral Pressure and Blood Pressure in anesthetized Dogs (KMD-0015-A)
Effects of KMD-3213 on Phenylephrine-Induced Increase of Intraurethral Pressure in Rats – Duration of Action (KMD-0021-A)
Effects on action potential parameters in the isolated guinea pig papillary muscle for KMD-3213.
Safety pharmacology studies of MD127K: effects on action potential parameters in isolated guinea pig
Safety pharmacology study of KMD-3213
Effect of KMD-3213 on hERG tail current recorded from stably transfected HEK293 cells
Safety pharmacology study of KMD-3213 oral administration on the dog respiratory system
Effects on blood hormone levels in rats by oral administration of KMD-3213 (KMD-TX2002-703E01).
Effects on blood hormone levels in mice by oral administration of KMD-3213 (KMD-TX2002-704E01)
Investigation of thyroid hypertrophy in rats by repeated oral administration of KMD-3213 (Study no. 10283).

Toxicology:

Toxicity to dogs by repeated capsule administration for 52 weeks.
Twenty-six-week oral toxicity study of KMD-3213 in rats
Additional 26-week oral toxicity study of KMD-3213 in rats
Thirteen week study in dogs
Single Dose Toxicity Study of KMD-3213 Administered Orally in Rats (00229)
Single Dose Toxicity Study of KMD-3213 Administered Orally in Beagle Dogs (00233)
One-Month toxicity Study of KMD-3213 Administered Orally Followed by a 1-Month recovery Study in Rats (00238)
Two-week oral toxicity study of KMD-3213 in Beagle dogs.
Preliminary study by oral administration of KMD-3213 for effects on embryo-fetal development in rats.
Preliminary study by oral administration of KMD-3213 for effects on embryo-fetal development in rabbits.
Preliminary study by oral administration of KMD-3213 for the effects on pre- and postnatal development,
Single dose intravenous toxicity study of 0.1 M citric acid buffer solution (pH 4.0) in rats
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
Reverse mutation test of KMD-3213 using bacteria
Reverse mutation test of MD127K in bacteria

Chromosomal aberration test of MD127K in cultured Chinese hamster cells.
Mammalian cell mutation assay
Chromosomal aberration test of KMD-3213 with mammalian cells in culture
Chromosomal aberration test of KMD-3213 with mammalian cells in culture (by short-term treatment without chromosomal activation)
Micronucleus study of KMD-3213 in mice
Rat liver DNA repair (UDS) test
Carcinogenicity Study by Dietary Administration to CD-1 Mice for 104 Weeks
Carcinogenicity Study by Dietary Administration to Male CD-1 Mice for 104 Weeks (replacement study)
Carcinogenicity Study by Dietary Administration to CD Rats for 104 Weeks
Intramuscular local irritation study in rabbits
Single dose intravenous toxicity study of 0.1 M citric acid buffer solution (pH 4.0) in rats
In vitro hemolysis study with human whole blood
Silodosin: Evaluation of *in vitro* phototoxicity on Balb/c 3T3 fibroblasts using neutral red assay.
Single dose oral (gavage) phototoxicity evaluation of silodosin in hairless mice
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

ADME:

^{14}C-KMD-3213: Absorption, metabolism, and excretion in the dog after single oral administration.
A Toxicokinetic Study of KMD-3213 in Rats After Single Oral Administration (10216)
Assumption of the enzyme responsible for the metabolism of KMD-3213 to its main metabolite KMD-3293
Absorption site of ^{14}C-KMD-3213 in male rat alimentary tract
Distribution of ^{14}C-KMD-3213 to rat blood cells (*in vitro* study)
Plasma concentration of radioactivity after single oral or intravenous administration of ^{14}C-KMD-3213 to male rats
Identification of the enzyme responsible for production of metabolite KMD-3310 from KMD-3213
In vitro plasma protein binding of KMD-3213 glucuronide
In vitro plasma protein binding of KMD-3293
In vitro metabolism study of ^{14}C-KMD-3213—identification of UGT isoforms using microsomes expressing human UGT and determination of Km and Vmax values
A toxicokinetic study of KMD-3213 in rats (Sprague-Dawley) after single a oral administration (10216).
A study of KMD-3213 metabolites in mice after a single oral administration (10235).
A study of KMD-3213 metabolites in dogs (male Beagle) after a single oral administration (10211).
Pharmacokinetic study of KMD-3213: tissue distribution of radioactivity in rats after a single oral administration of 14C-KMD-3213
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

Other:

KMD-3213: Intramuscular local irritation study in rabbits.
In vitro hemolysis study with human whole blood.
In Vitro Study of Inhibitory Effect on Trypsin
In Vitro Study of Inhibitory Effect on Papain

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Silodosin is an alpha-1 adrenoceptor antagonist.

In a receptor binding study using membrane fractions of mouse-derived LM (tk-) cells in which each of three human α1-adrenergic receptor (AR) subtypes (α_{1A}-, α_{1B}- and α_{1D}-ARs) was expressed, silodosin (KMD-3213) showed higher selectivity for the α_{1A}-AR subtype than either tamsulosin hydrochloride, prazosin hydrochloride or terazosin hydrochloride (other α_1-AR blockers). The affinity for the α_{1A}-AR subtype was highest with tamsulosin hydrochloride, followed by silodosin, prazosin hydrochloride and terazosin hydrochloride.

Silodosin showed a strong antagonism to noradrenaline-induced contraction in lower urinary tract tissues of rabbits including the prostate, urethra and trigone of bladder (α_{1A}-AR) with pA2 or pKb of 9.60, 8.71 and 9.35, respectively.

The pA2 of the antagonism to noradrenaline-induced contraction for silodosin in the isolated rat spleen (α_{1B}-AR) and isolated rat thoracic aorta (α_1D-AR) were 7.15 and 7.88, respectively. The selectivity of silodosin for the prostate was about 280-fold higher than that for spleen and about 50-fold higher than that for the thoracic aorta, indicating high selectivity of silodosin for the lower urinary tract tissues. Tamsulosin hydrochloride showed a high antagonism to noradrenaline-induced contraction in every tissue studied (pA2 or pKb: 8.64 – 9.93).

Intravenously injected silodosin inhibited phenylephrine-induced increases in urethral pressure in anesthetized rats in a dose-dependent manner and showed hypotensive effects at higher dosage. When its selectivity for the lower urinary tract was compared with that of other α_1-AR blockers, silodosin showed the highest selectivity, followed by tamsulosin hydrochloride, prazosin hydrochloride and terazosin hydrochloride. Silodosin, after intraduodenal administration, also inhibited phenylephrine-induced increases in urethral pressure in anesthetized rats in a dose-dependent manner, and showed hypotensive effects at higher dosage. Tamsulosin hydrochloride inhibited phenylephrine-induced increase in urethral pressure like silodosin; however, the selectivity for the lower urinary tract was higher for silodosin.

Silodosin (oral), at doses of 0.1, 1 and 3 mg/kg inhibited phenylephrine-induced increase in urethral pressure up to 12, 18 and 24 hours after administration, respectively. Tamsulosin hydrochloride orally-administered at a dose of 1 mg/kg did not show inhibitory effects at 18 hours after administration.

Intravenously-injected silodosin and tamsulosin hydrochloride inhibited urethral pressure increases induced by electrostimulation of the hypogastric nerve in anesthetized dogs and showed a hypotensive effect at higher dosage. The hypotensive effects of silodosin were weaker than that of tamsulosin hydrochloride, while silodosin selectivity for the lower urinary tract was significantly higher than that of tamsulosin hydrochloride.

Both silodosin and tamsulosin hydrochloride inhibited overactive bladder-like symptoms observed in the rat benign prostatic hyperplasia model induced by sex hormones.

The proposed mechanism of action is that silodosin blocks the sympathetic nervous system via the α_{1A}-AR subtype distributed in the prostate, urethra and trigone of the bladder to relieve tension of the smooth muscles of lower urinary tract tissues. This reaction decreases urethral pressure, thereby improving the symptoms associated with benign prostatic hyperplasia.

Prazosin hydrochloride and terazosin hydrochloride showed the highest affinity for α_{1B}-AR subtype, while silodosin showed the highest affinity for α_{1A}-AR subtype. The affinity for α_{1A}-AR subtype of tamsulosin hydrochloride was about 3-fold higher than that of silodosin. However, the ratio of the selectivity for α_{1A}-AR subtype to that for α_{1B}-AR of tamsulosin hydrochloride was about 10-fold, which was lower than that of silodosin at about 160-fold. The silodosin effect was persistent.

The main metabolite in animals (rats and dogs) was KMD-3310, while the glucuronide conjugate of silodosin (KMD-3213G) and KMD-3293 were identified as major human metabolites.

The affinities of KMD-3213G and KMD-3293 for human α_{1A}-AR subtype were 8- and 42-fold lower than the corresponding affinity of silodosin, respectively. KMD-3310, which was identified as the main metabolite in animals, showed no affinity for any α_1-AR subtypes. The affinities of the other identified metabolites, KMD-3241, KMD-3289 and KMD-3295, for α_{1A}-AR subtype were equal to or lower than that of silodosin.

The affinities of KMD-3213G for rat mandibular gland, where α_{1A}-AR subtype is mainly distributed, and for rat liver, where α_{1B}-AR subtype is mainly distributed, were 4.5- and 35- fold lower than the corresponding affinity of silodosin, respectively.

In isolated rat prostate tissue, both silodosin and KMD-3213G caused the concentration-response curve of noradrenaline-induced contractions to shift to the right, and treatment with the parent and metabolite at higher concentrations caused a decrease in the maximum contractile response. The pKb values of silodosin and KMD-3213G were 10.15 and 9.86, respectively, while the activity of KMD-3213G was about half of that of silodosin.

The plasma and prostate concentrations of silodosin and KMD-3213G were compared after intravenous injection in rats. After intravenous injection of silodosin, the concentration of silodosin was higher in the prostate than in plasma. After intravenous injection of KMD- 3213G, the concentration of this metabolite in the prostate was lower than the concentration in plasma. The ratio of prostate/plasma concentrations of KMD-3213G was about 19- and 10-fold lower than that of silodosin at 1 and 4 hours, respectively, after the start of injection. This indicates that KMD-3213G was less distributed in the prostate than silodosin.

Silodosin's affinity for $ß_2$-AR (pKi value: 8.25) was almost equal to that for α_{1D}-AR subtype (pKi value: 8.66) and α_{1B}-AR subtype (pKi: 8.19), but showed affinity only at higher concentrations for α_2- and $ß_1$-AR, muscarinic receptor, serotonin (5-HT1) receptor and dopamine (D1, D2 long, D3 and D4.2) receptors. The affinities of silodosin for any receptor subtypes other than α_{1A}-AR were lower than that for α_{1A}-AR.

A uterus specimen isolated from a pregnant rat was used to determine the action on $ß_2$-AR. Silodosin showed antagonism to $ß_2$-AR at concentrations of 3×10^{-6} mol/L and higher.

The affinity of KMD-3213G for dopamine D3 receptor was almost equal to that of silodosin, although its affinities for α_2-, $ß_1$- and $ß_2$-ARs, muscarinic receptor, serotonin (5-HT1) receptor and dopamine (D1, D2 long, D3 and D4.2) receptors were lower than the corresponding affinities of silodosin.

KMD-3293 showed a lower affinity for all tested receptors than did silodosin.

2.6.2.2 Primary pharmacodynamic study summaries

Mechanism of action, *in vitro* receptor binding, antagonism of smooth muscle contraction *in situ*, etc. for silodosin and its metabolites:

Affinity for Human α_1-AR Subtypes :

Report Title:	Receptor binding study of KMD-3213 and its metabolite and optical isomer	Study No.:	KMD-0005-A
Test Material:	Membrane fraction of LM(tk-) cells expressing human receptors	Test Article:	Silodosin
Route of Administration:	*In vitro*		

Drug	Concentration used (mol/L)			Results: pKi value		
	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR
silodosin (KMD-3213)	1×10^{-12} - 1×10^{-8} (Common ratio 10)	1×10^{-10} - 1×10^{-6} (Common ratio 10)	1×10^{-11} - 1×10^{-7} (Common ratio 10)	10.4 ± 0.07	8.19 ± 0.04	8.66 ± 0.02
tamsulosin hydrochloride	1×10^{-11} - 1×10^{-8} (Common ratio 10)	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-12} - 1×10^{-8} (Common ratio 10)	10.9 ± 0.07	9.92 ± 0.01	10.5 ± 0.07
prazosin hydrochloride	1×10^{-12} - 1×10^{-8} (Common ratio 10)	1×10^{-12} - 1×10^{-8} (Common ratio 10)	1×10^{-12} - 1×10^{-8} (Common ratio 10)	9.91 ± 0.03	10.6 ± 0.03	10.1 ± 0.04
terazosin hydrochloride	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-11} - 1×10^{-7} (Common ratio 10)	8.71 ± 0.06	9.40 ± 0.02	9.07 ± 0.01

Actions on Noradrenaline-Induced Contraction in the Isolated Rabbit Tissues

Report Title:	Effects of KMD-3213 on noradrenaline-induced contraction in the isolated rabbit prostate, urethra and trigone of bladder	Study No.:	KMD-0010-A
Species/strain/sex:	Rabbit/Japanese White/male	Test Article:	Silodosin
Route of Administration:	*In vitro*		

Test material	Drug	Concentration used (mol/L)	Results	
Prostate specimen	silodosin (KMD-3213)	1×10^{-9}, 1×10^{-8}	Concentration-dependent antagonism and inhibition of the maximum response by about 30% by treatment at 1×10^{-8} mol/L	pKb: 9.60 ± 0.05
	tamsulosin hydrochloride	1×10^{-9}, 1×10^{-8}	Concentration-dependent antagonism and inhibition of the maximum response by about 30% by treatment at 1×10^{-8} mol/L	pKb: 9.93 ± 0.07
	prazosin hydrochloride	1×10^{-9} - 3×10^{-7} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 7.91 ± 0.02
Urethra specimen	silodosin (KMD-3213)	3×10^{-9} - 1×10^{-7} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 8.71 ± 0.09
	tamsulosin hydrochloride	3×10^{-9} - 1×10^{-7} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 9.00 ± 0.06
	prazosin hydrochloride	3×10^{-9} - 1×10^{-5} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 7.95 ± 0.04
Trigone of bladder specimen	silodosin (KMD-3213)	3×10^{-9} - 1×10^{-7} (Common ratio 3)	Concentration-dependent antagonism and inhibition of the maximum response by about 40% by treatment at 1×10^{-7} mol/L	pKb: 9.35 ± 0.15
	tamsulosin hydrochloride	1×10^{-9} - 3×10^{-8} (Common ratio 3)	Concentration-dependent antagonism and inhibition of the maximum response by about 40% by treatment at 3×10^{-8} mol/L	pKb: 9.48 ± 0.14
	prazosin hydrochloride	3×10^{-9} - 1×10^{-5} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 8.10 ± 0.05

Actions on Noradrenaline-Induced Contraction in Isolated Rat Tissues

Report Title:	Effects of KMD-3213 on noradrenaline-induced contraction in the isolated rat thoracic aorta and spleen	Study No.:	KMD-0007-A
Species/strain/sex:	Rat/SD/male	Test Article:	Silodosin
Route of Administration:	*In vitro*		

Test material	Drug	Concentration used (mol/L)	Results	
Thoracic aorta specimen	silodosin (KMD-3213)	3×10^{-8} - 1×10^{-6} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 7.88 ± 0.05
	tamsulosin hydrochloride	1×10^{-9} - 3×10^{-8} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 9.82 ± 0.06
	prazosin hydrochloride	1×10^{-9} - 3×10^{-8} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 9.17 ± 0.06
Spleen specimen	silodosin (KMD-3213)	1×10^{-7} - 1×10^{-5} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 7.15 ± 0.05
	tamsulosin hydrochloride	3×10^{-9} - 1×10^{-7} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 8.64 ± 0.06
	prazosin hydrochloride	1×10^{-9} - 3×10^{-8} (Common ratio 3)	Concentration-dependent antagonism	pA_2: 9.34 ± 0.13

Effects in Rats *In Situ* – Intravenous Injection

Report Title:	Effects of KMD-3213 on blood pressure, heart rate and phenylephrine-induced increase in urethral pressure in rats: Intravenous injection	Study No.:	KMD-0006-A
Species/strain/sex:	Rat/SD/male	Test Article:	Silodosin
Route of Administration:	Intravenous injection		

Drugs	Dose (μg/kg)		Results		
			Increase in urethral pressure	Blood pressure [a)]	Selectivity for the lower urinary tract (ED_{15}/ID_{50})
	Increase in urethral pressure	Blood pressure [a)]	ID_{50} (μg/kg)	ED_{15} (μg/kg)	
silodosin (KMD-3213) [b)]	0.3 - 30 (Common ratio 3)	1 - 300 (Common ratio 3)	0.932	10.9	11.7
tamsulosin hydrochloride	0.03 - 3 (Common ratio 3)	0.1 - 30 (Common ratio 3)	0.400	0.895	2.24
prazosin hydrochloride	0.3 - 30 (Common ratio 3)	0.1 - 30 (Common ratio 3)	4.04	0.792	0.196
terazosin hydrochloride	3 - 300 (Common ratio 3)	1 - 300 (Common ratio 3)	38.7	6.60	0.171

a) Mean blood pressure.
b) KMD-3213·2HBr salt

Effects in Rats *In Situ* - Intraduodenal Injection

Report Title:	Effects of KMD-3213 on blood pressure, heart rate and phenylephrine-induced increase in urethral pressure in rats: Intraduodenal administration	Study No.:	KMD-0013-A
Species/strain/sex:	Rat/SD/male	Test Article:	Silodosin
Route of Administration:	Intraduodenal administration		

Drug	Dose (μg/kg)		Results		
			Increase in urethral pressure	Blood pressure [a)]	Selectivity for the lower urinary tract (ED_{15}/ID_{50})
	Increase in urethral pressure	Blood pressure [a)]	ID_{50} (μg/kg)	ED_{15} (μg/kg)	
silodosin (KMD-3213)	3 - 300 (Common ratio 3)	100 - 3000 (Common ratio 3)	10.6	276	26.0
tamsulosin hydrochloride	3 - 100 (Common ratio 3)	10 - 300 (Common ratio 3)	5.45	20.8	3.82

a) Mean blood pressure.

Effects in Rats *In Situ* – Persistency of Effect

Report Title:	Effect of KMD-3213 on phenylephrine-induced increase in urethral pressure in rats - Persistency of the effect	Study No.:	KMD-0021-A
Species/strain/sex:	Rat/SD/male	Test Article:	Silodosin
Route of Administration:	Oral administration		

Drug	Dose (μg/kg)	Time after drug treatment (hr)	Results
silodosin (KMD-3213)	30 - 3000 (Common ratio 3)	12	Significant suppression of phenylephrine-induced increase in urethral pressure as compared with the control group at 100 μg/kg or higher dose
		18	Significant suppression of phenylephrine-induced increase in urethral pressure as compared with the control group at 1000 μg/kg or higher dose
		24	Significant suppression of phenylephrine-induced increase in urethral pressure as compared with the control group at 3000 μg/kg
tamsulosin hydrochloride	30 - 3000 (Common ratio 3)	12	Significant suppression of phenylephrine-induced increase in urethral pressure as compared with the control group at 300 μg/kg or higher dose
		18	Significant suppression of phenylephrine-induced increase in urethral pressure as compared with the control group at 3000 μg/kg
		24	No significant suppression of phenylephrine-induced increase in urethral pressure observed as compared with the control group up to 3000 μg/kg

Effects in Dogs *In Situ*

Report Title:	Effects on hypogastric nerve stimulation-induced increase in urethral pressure and on blood pressure in anesthetized dogs	Study No.:	KMD-0015-A
Species/strain/sex:	Dogs/beagle/male	Test Article:	Silodosin
Route of Administration:	Intravenous injection		

Drug	Dose (μg/kg)	Results		
		Increase in urethral pressure	Blood pressure [a]	Selectivity for the lower urinary tract (ED_{15}/ID_{50})
		ID_{50} (μg/kg)	ED_{15} (μg/kg)	
tamsulosin hydrochloride	0.3 - 300 (Common ratio 3)	1.86 ± 0.479	440 ± 198	237 ± 76.6
silodosin (KMD-3213) [b]	0.3 - 300 (Common ratio 3)	0.908 ± 0.300	0.837 ± 0.130	1.21 ± 0.296

a) Mean blood pressure.
b) KMD-3213•2HBr salt.

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Affinity of Metabolites for Human α_1-AR Subtypes

Report Titles: Receptor binding study of KMD — Study No.: KMD-0005-A
Receptor binding study of the metabolite of KMD — KMD-0009-A
Test material: Membrane fraction of LM(tk-)cells expressing human receptors
Test Article: Silodosin and related metabolites
Route of administration: *In vitro*

Drug	Concentration used (mol/L)			Results: pKi		
	α_{1A}-AR	α_{1B}-AR	α_{1D}-AR	α_{1A}-AR	α_{1D}-AR	α_{1B}-AR
KMD-3213G [a]	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-8} - 1×10^{-4} (Common ratio 10)	1×10^{-7} - 1×10^{-3} (Common ratio 10)	9.50 ± 0.03	6.53 ± 0.10	7.09 ± 0.06
KMD-3241 [b]	1×10^{-12} - 1×10^{-8} (Common ratio 10)	1×10^{-10} - 1×10^{-6} (Common ratio 10)	1×10^{-10} - 1×10^{-6} (Common ratio 10)	10.5 ± 0.05	8.56 ± 0.01	9.16 ± 0.08
KMD-3289	1×10^{-12} - 1×10^{-8} (Common ratio 10)	1×10^{-10} - 1×10^{-6} (Common ratio 10)	1×10^{-10} - 1×10^{-6} (Common ratio 10)	10.2 ± 0.02	8.92 ± 0.01	8.87 ± 0.02
KMD-3293	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-8} - 1×10^{-4} (Common ratio 10)	1×10^{-8} - 1×10^{-4} (Common ratio 10)	8.78 ± 0.03	6.97 ± 0.02	7.13 ± 0.04
KMD-3295	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-8} - 1×10^{-4} (Common ratio 10)	1×10^{-8} - 1×10^{-4} (Common ratio 10)	8.97 ± 0.06	6.99 ± 0.04	7.42 ± 0.04
KMD-3310	1×10^{-7} - 1×10^{-4} (Common ratio 10)	1×10^{-7} - 1×10^{-4} (Common ratio 10)	1×10^{-7} - 1×10^{-4} (Common ratio 10)	< 5.0	< 5.0	< 5.0
KMD-3290 [c]	1×10^{-11} - 1×10^{-7} (Common ratio 10)	1×10^{-9} - 1×10^{-5} (Common ratio 10)	1×10^{-9} - 1×10^{-5} (Common ratio 10)	9.12 ± 0.02	7.44 ± 0.04	7.64 ± 0.10

a) Glucuronide conjugate Na of KMD-3213 (MD127NA or KMD-3213G).
b) KMD-3241 oxalate.
c) Optical isomer.

Affinity of Glucuronide Conjugate of Silodosin for Rat α_1-AR Subtypes

Report Title: Study on binding of KMD-3213 and MD127K to rat α_1-adrenoreceptors — Study No.: AL-2232-G
Species/strain/sex: Rat
Test Article: Silodosin and glucuronide conjugate K metabolite of silodosin (MD127K or KMD-3213G)
Route of Administration: *In vitro*

Test item	Test material	Drug	Concentration used (mol/L)	Results: pKi value
α_{1A}-AR	Rat mandibular gland	silodosin (KMD-3213)	1×10^{-12} - 1×10^{-7} (Common ratio 10)	9.92
		glucuronide conjugate K of KMD-3213	1×10^{-12} - 1×10^{-7} (Common ratio 10)	9.27
α_{1B}-AR	Rat liver	silodosin (KMD-3213)	1×10^{-9} - 1×10^{-4} (Common ratio 10)	7.39
		glucuronide conjugate K of KMD-3213	1×10^{-9} - 1×10^{-4} (Common ratio 10)	5.84

Effect of Glucuronide Conjugate of Silodosin on Noradrenaline-Induced Contraction in the Isolated Tissue

Report Title: Effects of KMD-3213 and MD127K on noradrenaline-induced contraction in the isolated rat prostate — Study No.: KMD-11004
Species/strain/sex: Rat/SD/male
Test Article: Silodosin and glucuronide conjugate K metabolite of silodosin (MD127K or KMD 3213G)
Route of Administration: *In vitro*

Test material	Drug	Concentration used (mol/L)	Results	
Prostate specimen	silodosin (KMD-3213)	1×10^{-9}, 1×10^{-8}	Concentration-dependent antagonism and inhibition of the maximum response by about 40% by treatment at 1×10^{-8} mol/L	pKb: 10.15 ± 0.04
	glucuronide conjugate K of KMD-3213	1×10^{-9}, 1×10^{-8}	Concentration-dependent antagonism and inhibition of the maximum response by about 25% by treatment at 1×10^{-8} mol/L	pKb: 9.86 ± 0.05

Distribution of Glucuronide Conjugate of Silodosin into the Prostate

Report Title: Measurement of plasma and prostatic concentrations after continuous injection of KMD-3213 and glucuronide conjugate of KMD-3213 in rats — Study No.: KMD-11005

Species/strain/sex: Rat/SD/male

Test Article: Silodosin and glucuronide conjugate K metabolite of silodosin (MD127K or KMD 3213G)

Route of Administration: Continuous intravenous injection

Drug	Dose (μg/kg/hr)	Measured Substance	Results			
			Plasma concentration (ng/mL)		Prostatic concentration (ng/g)	
			After 1-hr continuous injection	After 4-hr continuous injection	After 1-hr continuous injection	After 4-hr continuous injection
silodosin (KMD-3213) a)	120	silodosin (KMD-3213)	33.23 ± 5.89	40.98 ± 7.03	163.45 ± 172.60	297.17 ± 71.95
		KMD-3213G b)	N.D.	N.D.	1.54 ± 3.78 c)	5.12 ± 6.00 d)
glucuronide conjugate K of KMD-3213	120	silodosin (KMD-3213)	N.D.	N.D.	1.96 ± 4.79 e)	3.12 ± 4.86 e)
		KMD-3213G b)	114.24 ± 27.53	117.93 ± 37.86	26.08 ± 8.63	90.20 ± 53.63

Drug	Measured substance	Results			
		After 1-hr continuous injection		After 4-hr continuous injection	
		Prostatic/plasma concentration ratio f)	Relative ratio	Prostatic/plasma concentration ratio f)	Relative ratio
silodosin (KMD-3213) a)	silodosin (KMD-3213)	4.70 ± 4.14	1.0	7.55 ± 2.57	1.0
glucuronide conjugate K of KMD-3213	KMD-3213G b)	0.25 ± 0.10	1/18.8	0.73 ± 0.30	1/10.3

N.D.: Not detected (below the quantitative limit) (plasma concentration: <2.5 ng/mL, prostatic concentration: <1.0 ng/prostate).

a) KMD-3213 • 2HBr salt, b) Glucuronide conjugate of KMD-3213, c) N.D. in 5 of 6 animals, d) N.D. in 3 of 6 animals, e) N.D. in 4 of 6 animals, f) The prostatic/plasma concentration ratio means the ratio in terms of the measured substance.

Drug activity related to proposed indication:

The proposed mechanism of action is that silodosin blocks the sympathetic nervous system via the α_{1A}-AR subtype distributed in the prostate, urethra and trigone of the bladder to relieve tension of the smooth muscles of lower urinary tract tissues. This reaction decreases urethral pressure, thereby improving the symptoms associated with benign prostatic hyperplasia.

Effects on Rat Benign Prostatic Hyperplasia Model

Report Title: Effects of KMD-3213 on the urodynamics of sex hormone-induced benign prostatic hyperplasia model in rats — Study No.: KMD-11001

Species/strain/sex: Rat/SD/male — Test Article: Silodosin

Route of Administration: Intravenous injection

Drug	Dose (μg/kg)	Results
silodosin (KMD-3213) a)	1 - 100 (Common ratio 10)	Significant decrease in frequency of overactive bladder-like contraction as compared with the control group at 10 μg/kg or higher dose Significant decrease in the maximum intravesical pressure and bladder capacity as compared with the control group at 10 μg/kg
tamsulosin hydrochloride	1 - 100 (Common ratio 10)	Significant decrease in frequency of overactive bladder-like contraction and significant decrease in the maximum intravesical pressure as compared with the control group at 10 μg/kg or higher dose Significant decrease in bladder capacity as compared with the control group at 100 μg/kg

a) KMD-3213•2HBr salt.

2.6.2.3 Secondary pharmacodynamic study summaries:

In a battery of secondary binding studies, minimal effects were observed for silodosin and its metabolites.

Affinity for Various Receptors

Report title: Binding study using radioreceptor assay — Study No.: AL-1246
Test material: Rats and Sf9 cells — Test Article: Silodosin
Route of administration: *in vitro*

Test Item	Test Material	Results: Inhibition rate (%) Silodosin (KMD-3213) (mol/L)			
		1×10^{-7}	1×10^{-6}	1×10^{-5}	1×10^{-4}
α_2-AR	Rat cerebral cortex	1.33	13.31	47.45	86.89
β_1-AR	Rat cerebral cortex	13.06	48.60	84.25	100.00
Muscarine receptor	Rat cerebral cortex	0.00	9.34	0.00	20.21
Serotonin (5-HT_1) receptor	Rat striatum	21.63	40.42	72.25	94.04

Test item	Test material	Results	
		IC_{50} value (mol/L)	pKi value
β_2-AR	Sf9 cells expressing human receptor	6.93×10^{-9}	8.25
Dopamine D_1 receptor	Sf9 cells expressing human receptor	3.01×10^{-5}	4.82
Dopamine D_2 long receptor	Sf9 cells expressing human receptor	3.45×10^{-5}	5.53
Dopamine D_3 receptor	Sf9 cells expressing rat receptor	2.05×10^{-6}	6.46
Dopamine $D_{4.2}$ receptor	Sf9 cells expressing human receptor	7.85×10^{-6}	6.30

The result is presented by the inhibition rate for α_2-AR, β_1-AR, muscarine receptor and serotonin (5-HT_1) receptor among nine receptors, while that for β_2-AR and dopamine (D_1, D_2 long, D_3 and $D_{4.2}$) receptors is presented by pKi value.

Concentrations of silodosin (KMD-3213) used in the study:
1×10^{-7} - 1×10^{-4} mol/L (common ratio 10, α_2-AR and β_1-AR, muscarine receptor and serotonin (5-HT_1) receptor).
1×10^{-9} - 1×10^{-4} mol/L (common ratio 3, β_2-AR and dopamine D_2 long receptor).
1×10^{-8} - 1×10^{-4} mol/L (common ratio 3, dopamine (D_1, D_3 and $D_{4.2}$) receptors).

Pharmacological Action on β_2-AR

Report title: Effect of KMD-3213 on isolated pregnant rat uterus preparation — Study No.: KMD-0020-A
Species/strain/sex: Rats/SD/female — Test Article: Silodosin
Route of administration: *in vitro*

Test item	Concentration used (mol/L)	Results
Action of silodosin (KMD-3213) alone	1×10^{-8} - 1×10^{-5} (Common ratio 10)	No effect on oxytocin-induced rhythmic contraction up to 1×10^{-5} mol/L
Interaction with isoproterenol	3×10^{-7} - 1×10^{-5} (Common ratio 3)	Inhibition of relaxing effect of isoproterenol on oxytocin-induced rhythmic contraction at 3×10^{-7} mol/L or higher concentration

Affinity of Main Human Metabolite Glucuronide Conjugate of Silodosin for Various Receptors

Report title: Binding study to various receptors of MD127K — Study No.: AL-2233-G
Test material: Rats, Sf9 cells and CHO-K1 cells
Test Article: glucuronide conjugate K metabolite of silodosin (MD127K or KMD-3213G)
Route of administration: *in vitro*

Test item	Test material	Results: pKi value
		Glucuronide conjugate K of KMD-3213
α_2-AR	Rat cerebral cortex	N.D.
β_1-AR	Rat cerebral cortex	4.20
Muscarine receptor	Rat cerebral cortex	N.D.
Serotonin (5-HT_1) receptor	Rat striatum	N.D.
β_2-AR	Sf9 cells expressing human receptor	4.96
Dopamine D_1 receptor	Sf9 cells expressing human receptor	N.D.
Dopamine D_2 long receptor	Sf9 cells expressing human receptor	N.D.
Dopamine D_3 receptor	Sf9 cells expressing rat receptor	6.27
Dopamine $D_{4.2}$ receptor	CHO-K1 cells expressing human receptor	N.D.

N.D.: Not determined (the inhibition rate at the concentration of 1×10^{-4} mol/L was less than 50%).

Concentrations of glucuronide conjugate K of KMD-3213 used in the study:
1×10^{-7} - 1×10^{-4} mol/L (common ratio 10, α_2-AR and β_1-AR, muscarine receptor, serotonin (5-HT_1) receptor and dopamine (D_1, D_2 long, D_3 and $D_{4.2}$) receptors).
1×10^{-10} - 1×10^{-4} mol/L (common ratio 10, β_2-AR).

Binding of MD127K (glucuronide conjugate of silodosin) to various receptors:

α_2-adrenergic receptors

	Inhibition (%)							IC_{50} (M)
Compound (conc.)	$3x10^{-8}$	10^{-7}	$3x10^{-7}$	10^{-6}	$3x10^{-6}$	10^{-5}	10^{-4}	
MD127K	-	3.16	-	1.81	-	5.72	35.02	$>10^{4}$
Yohimbine	15.59	36.47	56.93	82.12	87.62	-	-	$2.11x10^{-7}$

β_1-adrenergic receptors

	Inhibition (%)								IC_{50} (M)
Compound (conc.)	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	-	0.00	0.00	9.37	55.53	$8.33x10^{-5}$
Propranolol	14.53	39.36	54.80	77.21	86.75	-	-	-	$7.43x10^{-6}$

β_2-adrenergic receptors

	Inhibition (%)									IC_{50} (M)
Compound	10^{-10}	$3x10^{-10}$	10^{-9}	$3x10^{-9}$	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	0.00	-	0.00	-	0.00	0.00	0.98	34.02	77.23	$2.25x10^{-5}$
Propranolol	15.50	26.69	47.85	77.47	92.92	-	-	-	-	$8.66x10^{-10}$

Dopamine D1 receptors

	Inhibition (%)									IC_{50} (M)
Compound	$3x10^{-10}$	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	-	-	0.00	0.00	2.28	7.55	$>10^{-4}$
SCH23390	13.16	30.83	49.57	67.52	77.37	-	-	-	-	$3.82x10^{-9}$

Dopamine D2 long (human) receptors

	Inhibition (%)								IC_{50} (M)
Compound	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	-	0.00	1.71	3.16	32.54	$>10^{-4}$
(+)-butaxclamol	4.18	19.12	47.58	74.57	96.08	-	-	-	$1.11x10^{-8}$

Dopamine D3 (rat) receptors

	Inhibition (%)								IC_{50} (M)
Compound	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	-	2.51	38.54	86.36	100	$1.58x10^{-6}$
(+)-butaxclamol	2.96	17.62	46.07	81.70	92.26	-	-	-	$1.02x10^{-8}$

Dopamine D4.2 (human) receptors

	Inhibition (%)								IC_{50} (M)
Compound	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	$3x10^{-7}$	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	4.03	-	9.53	5.63	11.82	$>10^{-4}$
haloperidol	15.11	27.67	42.81	65.17	76.49	-	-	-	$4.41x10^{-8}$

Muscarinic (non-selective) receptors

	Inhibition (%)									IC_{50} (M)
Compound	$3x10^{-10}$	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	-	-	1.93	0.88	3.12	0.00	$>10^{-4}$
atropine	10.60	14.83	25.77	57.81	77.66	-	-	-	-	$7.40x10^{-9}$

Serotonin 5HT1 (non-selective) receptors

	Inhibition (%)								IC_{50} (M)
Compound	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
MD127K	-	-	-	-	2.47	16.44	24.54	43.72	10^{-4}
serotonin	29.40	50.45	72.87	88.07	98.72	-	-	-	$2.90x10^{-9}$

Receptor	Kd (nM)	Bmax
β_1-adrenergic	1.62	5.60 fmol/mg tissue
β_2-adrenergic (human)	0.18	2302.75 fmol/mg protein
Dopamine D3 (rat)	1.03	6109.06 fmol/mg protein

Receptor	Substance	Ki (M)	pKi
β_1-adrenergic	MD127K	6.37×10^{-5}	4.20
β_1-adrenergic	propranolol	5.68×10^{-9}	8.25
β_2-adrenergic (human)	MD127K	1.09×10^{-5}	4.96
β_2-adrenergic (human)	propranolol	4.21×10^{-10}	9.38
Dopamine D3 (rat)	MD127K	5.37×10^{-7}	6.27
Dopamine D3 (rat)	(+)-butaclamol	3.47×10^{-9}	8.46

Affinity of Main Human Metabolite KMD-3293 for Various Receptors

Report title: Binding study to various receptors of MD127K Study No.: AL-2233-G
Test material: Rats, Sf9 cells and CHO-K1 cells
Test Article: Metabolite KMD-3293
Route of administration: *in vitro*

Test item	Test material	Results: pKi value
		KMD-3293
α_2-AR	Rat cerebral cortex	N.D.
β_1-AR	Rat cerebral cortex	5.46
Muscarine receptor	Rat cerebral cortex	N.D.
Serotonin (5-HT_3) receptor	Rat striatum	N.D.
β_2-AR	Sf9 cells expressing human receptor	5.04
Dopamine D_1 receptor	Sf9 cells expressing human receptor	N.D.
Dopamine D_2 long receptor	Sf9 cells expressing human receptor	5.19
Dopamine D_3 receptor	Sf9 cells expressing rat receptor	5.92
Dopamine $D_{4.2}$ receptor	CHO-K1 cells expressing human receptor	N.D.

N.D.: Not determined (the inhibition rate at the concentration of 1×10^{-4} mol/L was less than 50%).
Concentrations of KMD-3293 used in the study:
1×10^{-7} - 1×10^{-4} mol/L (common ratio 10, α_2-AR and β_1-AR, muscarine receptor, serotonin (5-HT_3) receptor and dopamine (D_1, D_2 long, D_3 and $D_{4.2}$) receptors).
1×10^{-10} - 1×10^{-4} mol/L (common ratio 10, β_2-AR).

Binding of KMD-3293 to various receptors:

α_2-adrenergic receptors

	Inhibition (%)							IC_{50} (M)
Compound (conc.)	3×10^{-8}	10^{-7}	3×10^{-7}	10^{-6}	3×10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	0.88	-	3.92	-	6.91	35.64	$>10^{4}$
Yohimbine	13.37	33.81	59.36	83.49	94.12	-	-	1.99×10^{-7}

β_1-adrenergic receptors

	Inhibition (%)								IC_{50} (M)
Compound (conc.)	10^{-9}	3×10^{-9}	10^{-8}	3×10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	-	0.00	33.22	60.75	92.64	4.31×10^{-6}
Propranolol	33.00	63.54	74.85	88.89	100	-	-	-	2.08×10^{-9}

β_2-adrenergic receptors

	Inhibition (%)									IC_{50} (M)
Compound	10^{-10}	$3x10^{-10}$	10^{-9}	$3x10^{-9}$	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	0.42	-	2.98	-	0.00	2.64	6.61	42.07	84.55	$1.44x10^{-5}$
Propranolol	6.47	21.30	54.72	73.26	93.35	-	-	-	-	$1.00x10^{-9}$

Dopamine D1 receptors

	Inhibition (%)									IC_{50} (M)
Compound	$3x10^{-10}$	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	-	-	0.00	0.00	5.06	15.06	$>10^{-4}$
SCH23390	10.38	25.65	39.13	60.77	83.60	-	-	-	-	$4.66x10^{-9}$

Dopamine D2 long (human) receptors

	Inhibition (%)								IC_{50} (M)
Compound	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	-	0.00	2.80	21.13	64.78	$4.83x10^{-5}$
(+)-butaxclamol	14.45	32.09	54.21	69.48	90.46	-	-	-	$9.03x10^{-9}$

Dopamine D3 receptors

	Inhibition (%)								IC_{50} (M)
Compound	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	-	1.99	28.28	74.97	92.12	$2.88x10^{-6}$
(+)-butaxclamol	15.44	28.98	47.16	62.85	84.23	-	-	-	$1.13x10^{-8}$

Dopamine D4.2 receptors

	Inhibition (%)								IC_{50} (M)
Compound	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	$3x10^{-7}$	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	1.73	-	3.83	15.11	38.42	$>10^{-4}$
haloperidol	18.46	34.15	48.14	76.29	87.07	-	-	-	$2.43x10^{-8}$

Muscarinic (non-selective) receptors

	Inhibition (%)									IC_{50} (M)
Compound	$3x10^{-10}$	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	-	-	1.43	5.02	0.83	0.00	$>10^{-4}$
atropine	4.41	16.73	38.77	59.45	81.00	-	-	-	-	$5.85x10^{-9}$

Serotonin 5HT1 (non-selective) receptors

	Inhibition (%)								IC_{50} (M)
Compound	10^{-9}	$3x10^{-9}$	10^{-8}	$3x10^{-8}$	10^{-7}	10^{-6}	10^{-5}	10^{-4}	
KMD-3293	-	-	-	-	0.18	0.00	0.00	22.15	$>10^{-4}$
serotonin	21.01	39.43	65.30	84.16	96.43	-	-	-	$4.68x10^{-9}$

Receptor	Kd (nM)	Bmax
β_1-adrenergic	2.56	4.25 fmol/mg tissue
β_2-adrenergic	0.38	3222.22 fmol/mg protein
Dopamine D2 long	0.24	8887.28 fmol/mg protein
Dopamine D3	1.08	7134.80 fmol/mg protein

Receptor	Substance	Ki (M)	pKi
β_1-adrenergic	KMD-3293	$3.48x10^{-6}$	5.46
β_1-adrenergic	propranolol	$1.75x10^{-9}$	8.76
β_2-adrenergic	KMD-3293	$9.12x10^{-6}$	5.04
β_2-adrenergic	propranolol	$6.33x10^{-10}$	9.20
Dopamine D2 long	KMD-3293	$6.48x10^{-6}$	5.19
Dopamine D2 long	(+)-butaclamol	$1.21x10^{-9}$	8.92
Dopamine D3	KMD-3293	$1.21x10^{-6}$	5.92
Dopamine D3	(+)-butaclamol	$4.75x10^{-9}$	8.32

2.6.2.4 Safety pharmacology

Neurological effects: Orally-administered silodosin(KMD-3213) showed no effects on the central nervous system of rats at doses up to 2 mg/kg. After administration of silodosin at 20 mg/kg, trembling, decreased awakening levels and decreased body temperatures were observed.

The glucuronide metabolite of silodosin, KMD-3213G (or MD127K) did not show any effect on the central nervous (rats) after intravenous injections up to the highest dose of 3 mg/kg.

Safety pharmacology study of MD127K intravenous administration on rat central nervous system (4262, 15 August 2003, GLP). MD127K was administered to male Sprague-Dawley rats (N=5/group) at 0, 0.3, 1, and 3 mg/kg iv. No effects on home cage observations, hand-held, open field activity, stimulus response, or nervous/muscle responses were observed at 10, 30, 60, 120, or 240 minutes after dosing.

Safety pharmacology study of KMD-3213 (3291, 8 September 2000). CNS effects were observed in male Wistar rats (N=5/group) and cardiovascular and respiratory effects were observed in conscious male Beagle dogs (N=5/group)(oral; 0, 0.2, 2, and 20 mg/kg).

In rats, no effects were observed on home cage measurement (body position, respiration, clonic involuntary movement, tonic involuntary movement, vocalization, ptosis), handling observations (reactivity, handling, ptosis, lacrimation, salivation, piloerection), open field activity (open field rearing, clonic involuntary movement, tonic involuntary movement, gait, ambulatory movement, arousal, occurrence of stereotype, abnormal behavior, defecation, urination), stimulus response (response to approach, response to touch, sound response, tail pinch response, pupillary reflex, pinna reflex, corneal reflex, righting reflex), nervous and muscle observations (abdominal tone, limb tone, forelimb grip strength, hindlimb grip strength, landing foot asplay) up to 2 mg/kg and in spontaneous activity up to 20 mg/kg

rats	Males (mg/kg/day)			
	0	0.2	2	20
Clonic involuntary movement	0	0	0	1 at 1 hr and 2 at 2 hrs
Body temperature				
__predose	37.5	37.5	37.5	37.5
__0.5 hours postdose	37.4	37.4	37.3	36.8**
__1 hour postdose	37.6	37.8	37.7	36.3**
__2 hours postdose	37.3	37.4	37.1	36.2**
__4 hours postdose	37.2	37.3	37.1	36.7**
__6 hours postdose	37.3	37.4	37.2	37.1

Cardiovascular effects: Orally-administered silodosin in conscious dogs decreased blood pressure at doses as low as 0.2 mg/kg. No effect on heart rate and ECG was observed at doses up to 20 mg/kg.

Silodosin inhibited HERG tail current with the IC_{50} value of 8.91×10-6 mol/L. It prolonged APD_{90} in the papillary muscle isolated from guinea pig by 6.4% at 1×10^{-6} mol/L and by 17.1% at 1×10^{-5} mol/L.

In dogs, no effects of silodosin were observed on PR or QRS interval.

dogs	Males (mg/kg/day)			
	0	0.2	2	20
Respiratory rate				
__predose	16	15	14	16
__0.5 hours postdose	16	16	16	18
__1 hour postdose	17	16	17	16
__2 hours postdose	19	19	21	21
__3 hours postdose	17	21	19	23*
__4 hours postdose	16	13	23*	21
__6 hours postdose	14	14	15	24*
__8 hours postdose	15	13	17	20
__24 hours postdose	16	15	14	17
Systolic blood pressure				
__predose	150	152	151	154
__0.5 hours postdose	148	145	129	122**
__1 hour postdose	154	137	124**	117**
__2 hours postdose	147	136	126*	118**
__3 hours postdose	149	139	126*	119**
__4 hours postdose	150	141	127*	118**
__6 hours postdose	151	143	129*	120**
__8 hours postdose	148	152	134	117**
__24 hours postdose	144	157	155	147
Mean blood pressure				
__predose	98	97	95	98
__0.5 hours postdose	93	92	83	80*
__1 hour postdose	98	85*	83**	77**
__2 hours postdose	95	89	87	78**
__3 hours postdose	97	94	87	78**
__4 hours postdose	98	92	87	75**
__6 hours postdose	97	92	87	78**
__8 hours postdose	93	94	90	77*
__24 hours postdose	92	101	99	101
Diastolic blood pressure				
__predose	69	69	67	68
__0.5 hours postdose	65	64	58	57
__1 hour postdose	68	58*	59*	55**
__2 hours postdose	68	64	65	57
__3 hours postdose	70	70	65	56*
__4 hours postdose	70	66	66	53**
__6 hours postdose	69	64	66	55*
__8 hours postdose	64	65	66	54*
__24 hours postdose	65	71	70	76
Heart rate				
__predose	77	69	70	75
__0.5 hours postdose	75	83	84	88
__1 hour postdose	81	79	89	87
__2 hours postdose	**84**	**91**	**101**	**96**
__3 hours postdose	78	93	93	89
__4 hours postdose	77	79	96	81

__6 hours postdose	82	78	82	84
__8 hours postdose	73	68	91	85
__24 hours postdose	74	78	74	84
QT interval				
__predose	243	222	214	215
__0.5 hours postdose	224	205	226	217
__1 hour postdose	218	211	215	227
__2 hours postdose	204	205	200	223
__3 hours postdose	209	203	203	221
__4 hours postdose	222	215	199	222
__6 hours postdose	233	219	216	229
__8 hours postdose	224	221	209	223
__24 hours postdose	211	218	215	212
QTc				
__predose	274	229	232	224
__0.5 hours postdose	252	230	254	258
__1 hour postdose	254	235	254	262
__2 hours postdose	230	242	254	269
__3 hours postdose	233	251	243	261
__4 hours postdose	245	228	249	248
__6 hours postdose	271	247	248	262
__8 hours postdose	236	222	248	255
__24 hours postdose	234	244	237	238

KMD-3213G did not show any effect on the cardiovascular (dogs) systems after intravenous injections up to the highest dose of 3 mg/kg. It also did not show any effect on HERG tail current and myocardial action potential waveform in the papillary muscle isolated from guinea pigs at concentrations up to 1×10^{-5} mol/L.

Effects on action potential parameters in the isolated guinea pig papillary muscle for KMD-3213 (BO30118, 23 July 2003, GLP). In isolated guinea pig papillary muscle, action potential was measured using the glass microelectrode method for the metabolite KMD-3213 (10^{-7}, 10^{-6}, and 10^{-5} M). Sotalol (3×10^{-5} M) was used as a positive control.

N=6	KMD-3213 (M)				Sotalol (M)
	0	10^{-7}	10^{-6}	10^{-5}	3×10^{-5}
resting membrane pot. (mV)	-93	-95	-95	-93	-95
action potential amp. (mV)	131	132	133	131	132
action pot. durat.(50% repol.)(msec)	134	136	140*	146*	157*
action pot. durat.(90% repol.)(msec)	160	161	172*	186*	191*
max. upstroke velocity (V/sec)	217	219	229	216	231

Effect of KMD-3213 on hERG tail current recorded from stably transfected HEK293 cells (DDZO1007, 18 August 2003, GLP). Using the whole-cell patch-clamp method, the effects of KMD-3213 (0.1, 0.3, 1, 3, and 10 uM)(N=4 cells)(in 0.1% DMSO) were recorded with the cell initially clamped at –80 mV, after stepping the membrane potential to +20 mV, and then at –50 mV (tail current). E-4031 (100 nM) was used as a positive control.

KMD-3213 (uM)	Tail current (% control)	Tail current (% control, vehicle corrected)
0.1	84.5±2.9	99.1±3.4
0.3	83.7±3.8	98.2±4.4
1	73.7±2.7*	86.5±3.1
3	69.3±2.3**	81.3±2.6
10	38.3±3.7**	45.0±4.3

Treatment	Tail current (% control)
0.1% DMSO	85.2±1.7
E-4031	20.4±6.9

The IC_{25} and IC_{50} values for KMD-3213 inhibition of hERG tail current were 3.72 and 8.91 μM, respectively.

Safety pharmacology study of MD127K intravenous administration on the dog cardiovascular system and respiratory system (4263, 15 August 2003, GLP). MD127K (0, 0.3, 1, and 3 mg/kg iv) was administered to conscious dogs (N=4) in which a cannula for blood pressure measurements was surgically implanted. ECG, blood pressure, heart rate and respiration rate were measured at 0.25, 0.5, 1, 2, and 4 hours and oxyhemoglobin saturation, oxygen partial pressure, carbon dioxide partial pressure, and blood pH were measured at 0.5, 1, 2, and 4 hours. No drug related effects were observed at any dose tested.

Safety pharmacology studies of MD127K: effects on action potential parameters in isolated guinea pig papillary muscle (BO30526, 2 September 2003, GLP). In isolated guinea pig papillary muscle, action potential was measured using the glass microelectrode method for the metabolite MD127K (10^{-7}, 10^{-6}, and 10^{-5} M). Sotalol (3 x 10^{-5} M) was used as a positive control. No effect of MD127K at any concentration was observed on resting membrane potential, action potential amplitude, action potential duration at 50% or 90% repolarization, or maximal upstroke velocity. The positive control responded as expected.

Safety pharmacology studies of MD127K: effects of hERG current (BO31159, 24 December 2003, GLP). Using the whole-cell patch-clamp method, the effects of MD-127K (10^{-7}, 10^{-6}, and 10^{-5} M)(in 0.1% DMSO) were investigated in HEK293 cells stably expressing the hERG channel. E-4031 (100 nM) was used as a positive control. No significant effect of MD-127K was observed at the highest dose tested (10 uM). The positive control responded as expected.

Pulmonary effects: Orally-administered silodosin increased the respiratory rates in conscious dogs at a dose as low as 2 mg/kg, though it did not show any effect on blood gas parameters even at the highest dose of 20 mg/kg.

KMD-3213G did not show any effect on the respiratory (dogs) system after intravenous injections up to the highest dose of 3 mg/kg.

Safety pharmacology study of KMD-3213 oral administration on the dog respiratory system (3930, 20 September 2002, GLP). KMD-3213 was administered orally to male Beagle dogs (N=6) at 0, 0.2, 2, and 20 mg/kg. No effects on oxyhemoglobin saturation, oxygen partial pressure, carbon dioxide partial pressure, or blood pH were observed.

Renal effects: No studies were submitted.

Gastrointestinal effects: No studies were submitted.

Abuse liability: No studies were submitted.

Hormonal effects:

Effects on blood hormone levels in rats (Sprague-Dawley) by oral administration of KMD-3213 (KMD-TX2002-703E01)(9/4/00).

N=5	Males (mg/kg/day)					Females (mg/kg/day)				
	0	5	15	50	150	0	5	15	50	150
Prolactin (ng/ml)										
__after a single dose	1.82	2.69	10.6	24.0	25.2	7.92	21.8	38.4	103	191
__after 14 days	17.0	42.4	10.8	46.3	29.9	7.17	15.4	36.9	78.5	206
Progesterone (ng/ml)										
__after a single dose	-	-	-	-	-	4.65	2.67	2.60	2.48	1.89
__after 14-24 days						2.89	3.14	2.76	2.96	8.45
Estradiol (pg/ml)										
__after a single dose	-	-	-	-	-	0	0	0	0	0
__after 14-24 days						0	0	0	0	0
Estrus cycle prolongation or disappearance	-	-	-	-	-	0	0	0	3	5

Effects on blood hormone levels in mice (Slc:ICR) by oral administration of KMD-3213 (KMD-TX2002-704E01)(10/26/00).

N=5	Males (mg/kg/day)					Females (mg/kg/day)				
	0	5	15	50	150	0	6	20	60	200
Prolactin (ng/ml)										
__after a single dose	52.7	50.0	43.3	82.3	45.7	31.4	40.9	90.4	187	175
__after 14 days	28.1	46.5	50.1	43.5	54.0	51.3	40.3	93.8	166	238
Estrus cycle prolongation or disappearance	-	-	-	-	-	2	1	1	4	5

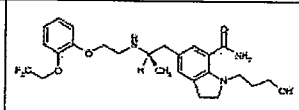
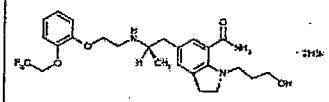
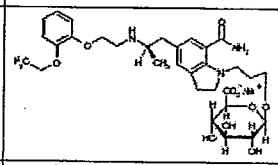
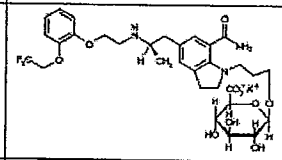
Investigation of thyroid hypertrophy in rats by repeated oral administration of KMD-3213 (Study no. 10283). KMD-3213 was administered to rats (Slc:SD, SPF, 10/sex/group) at doses of 0, 150 and 300 mg/kg for 4 weeks.

N=10	0 mg/kg (veh.)	150 mg/kg	300 mg/kg
TSH (ng/ml)	98.84	89.80	69.26
T3 (ng/ml)	2.54	2.50	3.01
T4 (ug/ml)	10.47	10.99	12.13*
Liver weight (g)	9.48	10.68**	11.37**
Relative liver weight (g/kg BW)	31.88	36.42**	40.96**
Thyroid weight (g)	0.018	0.018	0.019
Relative thyroid weight (g/kg BW)	0.59	0.63	0.69
Hypertrophy of thyroid follicular cells	0	1	2
Liver microsomal protein content (mg/g liver)	31.7	37.0**	41.7**
T4-glucuronosyltransferase activity (pmol/mg prot./min)	7.28	9.29**	11.40**

2.6.2.5 Pharmacodynamic drug interactions: NA

2.6.3 PHARMACOLOGY TABULATED SUMMARY

List of compounds in pharmacology studies:

Abbreviation Generic name	Chemical name	Chemical Structure	Origin
KMD-3213 (silodosin)	1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl} amino)propyl]-2,3-dihydro-1*H*-indole-7-carboxamide		Drug substance
KMD-3213 •2HBr salt	1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy) phenoxy]-ethyl} amino)propyl]-2,3-dihydro-1*H*-indole-7-carboxamide dihydrobromide		Dibromid hydroacid salt of the drug substance
MD127Na (glucuronide conjugate of silodosin Na)	Sodium (3-{7-carbamoyl-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl} amino) propyl]-2,3- dihydro-1*H*-indol-1-yl}propyl β-D-glucopyranosid) uronate		Sodium salt of metabolite
MD127K (glucuronide conjugate of silodosin K, or KMD-3213G)	Potassium (3-{7-carbamoyl -5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl} amino)propyl]-2,3- dihydro-1*H*-indol-1-yl}propyl β-D- glucopyranosid) uronate		Potassium salt of metabolite

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ON ORIGINAL

Type of Study	GLP*	Test System	Method of Administration	Testing Facility	Study Number
Primary Pharmacodynamics					
Receptor binding study of KMD-3213 and its metabolite and optical isomer	No	Mouse-derived LM(tk-) cells	*in vitro*	Kissei [a)]	KMD-0005-A
Effects of KMD-3213 on noradrenaline-induced contraction in the isolated rabbit prostate, urethra and trigone of bladder	No	Rabbits	*in vitro*	Kissei [a)]	KMD-0010-A
Effects of KMD-3213 on noradrenaline-induced contraction in the isolated rat thoracic aorta and spleen	No	Rats	*in vitro*	Kissei [a)]	KMD-0007-A
Effects of KMD-3213 on blood pressure, heart rate and phenylephrine-induced increase in urethral pressure in rats: Intravenous injection	No	Rats	Intravenous	Kissei [a)]	KMD-0006-A
Effects of KMD-3213 on blood pressure, heart rate and phenylephrine-induced increase in urethral pressure in rats: Intraduodenal administration	No	Rats	Intraduodenal	Kissei [a)]	KMD-0013-A
Effect of KMD-3213 on phenylephrine-induced increase in urethral pressure in rats - Persistency of the effect	No	Rats	Forced oral	Kissei [a)]	KMD-0021-A
Effects on hypogastric nerve stimulation-induced increase in urethral pressure and on blood pressure in anesthetized dogs	No	Dogs	Intravenous	Kissei [a)]	KMD-0015-A
Effects of KMD-3213 on the change in urodynamics in sex hormone-induced rat benign prostatic hyperplasia model	No	Rats	Intravenous	Kissei [a)]	KMD-11001
Receptor binding study of the metabolite of KMD-3213 (MD127Na)	No	Mouse-derived LM(tk-) cells	*in vitro*	Kissei [a)]	KMD-0009-A
Study on binding of KMD-3213 and MD127K to rat α1-adrenoreceptors	No	Rats	*in vitro*	—	AL-2232-G
Effects of KMD-3213 and MD127K on noradrenaline-induced contraction in the isolated rat prostate	No	Rats	*in vitro*	Kissei [a)]	KMD-11004
Measurement of plasma and prostatic concentrations after continuous injection of KMD-3213	No	Rats	Continuous intravenous injection	Kissei [a)]	KMD-11005
Secondary Pharmacodynamics					
Binding study using radioreceptor assay	No	Rats, Sf9 cells	*in vitro*	—	AL-1246
Effect of KMD-3213 on the uterus specimen isolated from pregnant rats	No	Rats	*in vitro*	Kissei [a)]	KMD-0020-A
Binding study to various receptors of MD127K	No	Rats, Sf9 cells, CHO-K1 cells	*in vitro*	—	AL-2233-G
Binding study to various receptors of KMD-3293	No	Rats, Sf9 cells, CHO-K1 cells	*in vitro*	—	AL-2336-G
Safety Pharmacology					
Effect of KMD-3213 on HERG Tail Current Recorded from Stably Transfected HEK293 Cells	Yes	HEK293 cells	*in vitro*	—	DDZO1007
Safety pharmacology study of KMD-3213 on action potential in the papillary muscle isolated from guinea pigs	Yes	Guinea pigs	*in vitro*	—	B030118
Safety pharmacology study of KMD-3213	No	Rats, Dogs	Forced oral	—	3291
Safety pharmacology study of KMD-3213 on the respiratory system after oral administration in dogs	Yes	Dogs	Forced oral	—	3930
Safety pharmacology study of MD127K on hERG tail current	Yes	HEK293 cells	*in vitro*	—	B031159
Safety pharmacology study of MD127K on action potential in the papillary muscle isolated from guinea pigs	Yes	Guinea pigs	*in vitro*	—	B030526
Safety pharmacology study of MD127K on the central nervous system after intravenous injection in rats	Yes	Rats	Intravenous	—	4262
Safety pharmacology study of MD127K on the cardiovascular and respiratory systems after intravenous injection in dogs	Yes	Dogs	Intravenous	—	4263
Pharmacodynamic Drug Interactions					
N/A					

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Safety Pharmacology Studies of Silodosin

Organ Systems	Species / Strain	Method of Administration	Doses (mg/kg) a)	Gender and No. per Group	Noteworthy Findings	GLP*	Study Number
Central nervous system	Rats / Wistar	Forced oral	0.2, 2, 20 (mg/kg)	10M	Decreased arousal level, decreased body temperature and tremors at 20 mg/kg group	No d)	3291
Respiratory system	Dogs / beagle	Forced oral	0.2, 2, 20 (mg/kg)	5M	No effect on hemoglobin O_2 saturation and other blood gas parameters (O_2 and CO_2 partial pressures, and blood pH)	Yes	3930
Cardiovascular system (including respiratory system)	Dogs / beagle	Forced oral	0.2, 2, 20 (mg/kg)	5M	Dosage-dependent decrease in blood pressure at 0.2 mg/kg and higher, and a transient increase in respiratory rate at 2 mg/kg or higher dose No effect on heart rate and ECG	No d)	3291
HERG b) tail current	HEK293 cells expressing HERG channel	*in vitro*	1×10^{-7} - 1×10^{-5} (mol/L) (Common ratio 3)	4	Inhibition of HERG tail current at 1×10^{-6} mol/L or higher concentration IC_{50} value: 8.91×10^{-6} mol/L	Yes	DDZO1007
Myocardial action potential waveform	Guinea pigs / Hartley	*in vitro*	1×10^{-7} - 1×10^{-5} (mol/L) (Common ratio 10)	6M	Prolonged APD_{90} c) at 1×10^{-5} mol/L and higher concentration 1×10^{-6} mol/L: 6.4% 1×10^{-5} mol/L: 17.1%	Yes	B030118

* An entry of "Yes" indicates that the study includes a GLP compliance statement.

a) Single administration unless otherwise provided.

b) HERG: Human ether-á-go-go related gene.

c) 90% repolarization action potential duration.

d) Reason for non-applicability: This study was conducted before the issue of "Guidelines for safety pharmacology studies (Notification No. 902 from Evaluation and Licensing Division, Pharmaceutical and Food Safety Bureau, MHLW as of June 21, 2002).

Safety Pharmacology Studies of Glucuronide Conjugate of Silodosin

Test Article: glucuronide conjugate K metabolite of silodosin (referred to as MD127K or KMD-3213G)

Organ Systems	Species / Strain	Method of Administration	Doses (mg/kg) a)	Gender and No. per Group	Noteworthy Findings	GLP*	Study Number
Central nervous system	Rats / SD	Intravenous injection	0.3, 1, 3 (mg/kg)	5M	No effect	Yes	4262
Respiratory system	Dogs / beagle	Intravenous injection	0.3, 1, 3 (mg/kg)	4M	No effect on respiratory rate, hemoglobin O_2 saturation and other blood gas parameters (O_2 and CO_2 partial pressures, and blood pH)	Yes	4263
Cardiovascular system (including respiratory system)	Dogs / beagle	Intravenous injection	0.3, 1, 3 (mg/kg)	4M	No effect on blood pressure, heart rate and ECG	Yes	4263
HERG b) tail current	HEK293 cells expressing HERG channel	*in vitro*	1×10^{-7} - 1×10^{-5} (mol/L) (Common ratio 10)	5	No effect	Yes	B031159
Myocardial action potential waveform	Guinea pigs / Hartley	*in vitro*	1×10^{-7} - 1×10^{-5} (mol/L) (Common ratio 10)	6M	No effect	Yes	B030526

* An entry of "Yes" indicates that the study includes a GLP compliance statement.

a) Single administration unless otherwise provided.

b) HERG: Human ether-á-go-go related gene.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Non-labeled silodosin and ^{14}C-KMD-3213 were used in absorption, distribution, metabolism and excretion studies in mice, rats, and dogs (ICR mice, Sprague-Dawley (SD) rats and Beagle dogs). The *in vitro* metabolism study in monkeys used hepatocytes from Cynomolgus monkeys. Male animals were selected for the pharmacokinetic studies and were administered drug orally and intravenously.

2.6.4.2 Methods of Analysis

Concentrations of silodosin and its metabolites in plasma and tissues were determined by high performance liquid chromatography (HPLC) fluorescent or liquid chromatography tandem mass spectrometry (LC/MS/MS) after extraction from measured samples using a [redacted] or an [redacted] The analytical method was validated for intra-assay and inter-assay reproducibility and if necessary, specificity, dilution effect, freeze/thaw stability and storage stability. b(4)

Radioactivity in samples was determined using a liquid scintillation counter for studies using ^{14}C-KMD-3213. The [redacted] method or the [redacted] method using a [redacted] was used as a pre-treatment method, when necessary. b(4)

In the metabolite determination study using ^{14}C-KMD-3213, the amount of each metabolite of silodosin was determined using HPLC with a radioactivity detector, and metabolites were measured by liquid chromatography / mass spectrometry (LC/MS).

Analytical Methods and Validation Reports

Species	Measurement Method	Sample and Its Amount Used (mL)	Target of Measurement	Range of Calibration Curve (ng/mL)	LLOQ (ng/mL)	Storage Stability of Samples		Study Site	Study No.
						Storage Temp.	Storage Period		
Mice (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin KMD-3241 KMD-3289 KMD-3293 KMD-3295	50-5000	50	-80°C	13 days	Kissei	10235
Mice (TK)	Fluorescence HPLC	Plasma[a] (0.1)	silodosin	10-10000	10	-20°C	25 days	[redacted]	KSI 086/982491 KSI 100/012988 KSI 114/012990
Mice	LC/MS/MS	Plasma	silodosin KMD-3241 KMD-3289 KMD-3293 KMD-3295	1.25-160 1.25-160 1.25-160 2.50-160 2.50-160	1.25 1.25 1.25 2.50 2.50	-80°C	31 days (KMD-3295, 165 days)	[redacted]	06-8934 06-8935b
Rats	LC/MS/MS	Plasma[a] (0.5)	silodosin	0.1-100	0.1	-20°C	4 weeks	Kissei	PK10054
Rats	LC/MS/MS	Plasma	silodosin KMD-3241 KMD-3289 KMD-3310 KMD-3293	1.25-160 1.25-160 1.25-160 2.50-160 2.50-160	1.25 1.25 1.25 2.50 2.50	-80°C	16 days (KMD-3310, 14 days)	[redacted]	06-8930 068931
Rats (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin KMD-3241 KMD-3289 KMD-3293 KMD-3295	50-5000	50	-80°C	2 weeks	Kissei	10216 10242 10308

a) [redacted] added, b) Glucuronide conjugate of silodosin. Kissei: Kissei Pharmaceutical Col, Ltd., [redacted]

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Species	Measurement Method	Sample and Its Amount Used (mL)	Target of Measurement	Range of Calibration Curve (ng/mL)	LLOQ (ng/mL)	Storage Stability of Samples		Study Site	Study No.
Rats (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin	10-10000	10	-80°C	3 months	Kissei	10026 10081 10092
Rats (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin	2-20	2	NA	NA	Kissei	10111
Rats (TK)	Fluorescence HPLC	Plasma (0.2)	MD127[b]	50-5000	50	-80°C	3 weeks	Kissei	10299 10308
Rats (TK)	Fluorescence HPLC	Plasma[a] (0.1)	silodosin	10-10000	10	-20°C	28 days	—	KSI 084/982477 KSI 102/012989
Rabbits (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin	10-10000	10	-80°C	30 days	Kissei	10116
Dogs	LC/MS/MS	Plasma	silodosin KMD-3241 KMD-3289 KMD-3310 KMD-3293 KMD-3295	1.25-160 2.50-160 1.25-160 2.50-160 2.50-160 2.50-160	1.25 2.50 1.25 2.50 2.50 2.50	-80°C	24 days (KMD-3295, 18 days and KMD-3310, 15 days)	—	06-8925c 06-8926 068927
Dogs	Fluorescence HPLC	Plasma[a] (1)	silodosin KMD-3241 KMD-3289	0.5-50 0.3-30 0.1-10	0.5 0.3 0.1	-40°C	1 month	Kissei	KMD-YA-7
Dogs	Fluorescence HPLC	Plasma[a] (1)	silodosin KMD-3241 KMD-3289	0.5-50 0.3-30 0.1-10	0.5 0.3 0.1	-20°C -20°C -20°C	1 year 1 year 6 months	Kissei	PK10075[a]

a) —) added, b) Glucuronide conjugate of silodosin, c) Only storage stability test was conducted.
NA – Not applicable.
Kissei: Kissei Pharmaceutical Col, Ltd., —

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Species	Measurement Method	Sample and Its Amount Used (mL)	Target of Measurement	Range of Calibration Curve (ng/mL)	LLOQ (ng/mL)	Storage Stability of Samples		Study Site	Study No.
Dogs	Fluorescence HPLC	Plasma[a] (1)	silodosin KMD-3241 KMD-3289	0.5-100 0.3-30 0.1-10	0.5 1.2 0.1	NA	NA	—	KSI 137/013760
Dogs (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin KMD-3241 KMD-3289 KMD-3293 KMD-3295	50-5000	50	-80°C	3 weeks (1 week only for KMD-3289)	Kissei	10221 10236
Dogs (TK)	Fluorescence HPLC	Plasma (0.2)	silodosin	10-10000	10	-80°C	1 month	Kissei	10008 10093
Dogs (TK)	Fluorescence HPLC	Plasma[a] (0.2)	silodosin	10-10000	10	-20°C	83 days	—	KSI 70/970908 KSI 71/974423
Dogs (TK)	Fluorescence HPLC	Plasma[a] (1)	silodosin KMD-3241 KMD-3289	10-10000 5-600 2-500	10 5 2	-20°C	34 days	—	KSI 115/994798
Dogs (TK)	Fluorescence HPLC	Liver[a] (1 g)	silodosin KMD-3241 KMD-3289	10-10000 ng/g 5-600 ng/g 2-500 ng/g	10 ng/g 5 ng/g 2 ng/g	-20°C	334 days (silodosin and KMD-3289) 226 days (KMD-3241)	—	KSI 115/994798
Dogs (TK)	Fluorescence HPLC	Kidney[a] (1 g)	silodosin KMD-3241 KMD-3289	10-10000 ng/g 5-600 ng/g 2-500 ng/g	10 ng/g 5 ng/g 2 ng/g	-20°C	200 days (silodosin and KMD-3241) 27 days (KMD-3289)	—	KSI 115/994798

a) —) added, b) Glucuronide conjugate of silodosin, c) Only storage stability test was conducted.
NA – Not applicable.
Kissei: Kissei Pharmaceutical Col, Ltd., —

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2.6.4.3 Absorption

Pharmacokinetics: Absorption after a Single Dose

Rats: Dose of Non-Labeled Compound Test Article: Silodosin

In fasting rats given a single oral dose of 0.3, 1 and 3 mg/kg silodosin, the tmax in plasma was 0.10 - 0.15 hours. The Cmax and AUC increased with increasing dose to 1 mg/kg, but was not dose proportional at higher levels. In non-fasting rats given a single intravenous injection of 0.03, 0.1, 0.3 and 1 mg/kg silodosin, the AUC increased with increasing dose over a range of 0.03 to 0.3 mg/kg. The $t_{1/2}$ of silodosin in rats after a single oral administration or intravenous injection was 1.5 to 2.0 hours and 2.4 to 3.2 hours, respectively. The bioavailability (BA) of silodosin in rats receiving a single oral administration of 0.3 and 1 mg/kg was about 9%. The CLtot of silodosin in single intravenous administration of silodosin to rats was 55 to 72 mL/min/kg.

Species/strain	Rats/SD			Rats/SD			
Sex (M/F)/N=	M/4			M/4			
Feeding	Fasting			Non-fasting			
Vehicle/dosing form	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)			Physiological saline (dissolved in dilute hydrochloric acid)			
Administration method	Forced oral	Forced oral	Forced oral	Intravenous	Intravenous	Intravenous	Intravenous
Dose (mg/kg)	0.3	1	3	0.03	0.1	0.3	1
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Substance to be assayed (target substance)	silodosin	silodosin	silodosin	silodosin	silodosin	silodosin	silodosin
Assay method	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS	LC/MS/MS
PK parameters:							
T_{max} (hr)	0.10 ± 0.04	0.10 ± 0.04	0.15 ± 0.04	–	–	–	–
C_{max} (ng/mL)	9.3 ± 7.1	28.7 ± 3.4	202.0 ± 153.4	–	–	–	–
AUC_{0-t} (ng·hr/mL)	6.9 ± 4.0 a)	27.9 ± 5.9 a)	170.0 ± 55.9 a)	7.2 ± 1.2	24.1 ± 5.2	77.5 ± 4.0	310.1 ± 54.3
$t_{1/2}$ (hr)	–	1.5 b)	2.0 ± 0.4 c)	3.2 ± 1.4	2.8 ± 0.3	2.8 ± 0.5	2.4 ± 0.9
CLtot (mL/min/kg)	–	–	–	71.2 ± 11.2	71.7 ± 15.3	64.6 ± 3.5	55.1 ± 10.1
Vd (L/kg)	–	–	–	11.4 ± 3.2	8.3 ± 2.6	6.3 ± 1.1	4.7 ± 2.1
BA (%)	9.4 ± 5.3	9.2 ± 1.7	–	–	–	–	–
Study No.	PK10066			PK10053			

a) AUC_{0-t} (AUC from time 0 to the final time point of concentration measurement), b) Mean of 2 cases, c) Mean of 3 cases.

-: Not calculated.

Data represents mean ± S.D.

Note: CLtot and Vd presented are results after intravenous injection.

Rats: Dose of Labeled Compound Test Article: Silodosin

Species	Rats/SD	Rats/SD	Rats/SD
Sex (M/F)/N=	M/3	M/3	M/3
Feeding	Fasting	Non-fasting	Fasting
Vehicle/dosing form	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)	Physiological saline (dissolved in dilute hydrochloric acid)
Administration method	Forced oral	Forced oral	Intravenous
Dose (mg/kg)	1	1	1
Sample	Plasma	Plasma	Plasma
Target substance to be assayed	TRA	TRA	TRA
Assay method	LSC	LSC	LSC
PK parameters:			
T_{max} (hr)	0.4	8.0	–
C_{max} (ng Eq/mL)	86.9	13.1	–
$AUC_{0-\infty}$ (ng eq.·hr/mL)	718.8	537.0	2564.1
$t_{1/2}$ (hr)	17.8	29.3	21.3
Study No.	PK10152		

LSC: Liquid scintillation counter.

TRA: Total radioactivity.

-: Not calculated, Data represents mean.

Bioavailability of KMD-3213 in dogs (KMD - YA-8) In fasting dogs receiving a single oral administration of 0.5 mg/kg silodosin, the plasma concentration reached Cmax within 1 hour and the $t_{1/2}$ was 2 hours. The BA in dogs after a single oral administration was about 25% and CLtot was 22.3 mL/min/kg.

Species/strain	Dogs/beagle			Dogs/beagle		
Sex (M/F)/N=	M/6			M/6		
Feeding	Fasting			Fasting		
Vehicle/dosing form	Filled in capsule (powder)			2HBr salt of KMD-3213 dissolved in physiological saline		
Administration method	Forced oral			Intravenous		
Dose (mg/kg)	0.5			0.5 c)		
Sample	Plasma			Plasma		
Target substance to be assayed	silodosin	KMD-3241	KMD-3289	silodosin	KMD-3241	KMD-3289
Assay method	HPLC	HPLC	HPLC	HPLC	HPLC	HPLC
PK parameters:						
T_{max} (hr)	0.88 ± 0.63	0.88 ± 0.63	1.08 ± 0.49	–	–	–
C_{max} (ng/mL)	37.58 ± 6.93	12.72 ± 3.19	5.07 ± 1.08	–	–	–
$AUC_{0-\infty}$ (ng·hr/mL)	97.3 ± 23.4	36.9 ± 9.6	10.2 ± 3.4	384.5 ± 71.6	46.8 ± 14.2	14.1 ± 4.0
$t_{1/2}$ (hr)	2.0 ± 0.3	2.3 ± 0.3	1.4 ± 0.4	3.3 ± 2.3	2.7 ± 0.5	2.1 ± 0.4
CLtot (mL/min/kg)	–	–	–	22.3 ± 4.3 a)	–	–
Vd (L/kg)	–	–	–	3.1 ± 1.2 b)	–	–
BA (%)	25.2 ± 2.9	–	–	–	–	–
Study No.	KMD-YA-8					

a) The unit was converted from mL/hr/kg to mL/min/kg.
b) The unit was converted from mL/kg to L/kg.
c) silodosin equivalent dose, -: Not calculated. -: Data represents mean ± S.D.
Note: CLtot and Vd presented are results after intravenous injection.

Dogs: ^{14}C-KMD-3213: Absorption, metabolism, and excretion in the dog after single oral administration (0.5 mg/kg)(KSI 137/013760, 2 August 2000). Test Article: Silodosin

Species/strain	Dogs/beagle			
Sex (M/F)/N=	M/3			
Feeding	Fasting			
Vehicle/dosing form	Filled in capsule (dissolved in dilute hydrochloric acid)			
Administration method	Forced oral			
Dose (mg/kg)	0.5			
Sample	Plasma			
Substance to be assayed (target substance)	silodosin	KMD-3241	KMD-3289	TRA
Assay method	HPLC	HPLC	HPLC	LSC
PK parameters:				
T_{max} (hr)	1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.7
C_{max} (ng/mL)	33.12 ± 6.90	6.64 ± 0.79	1.29 ± 0.12	318 a)
$AUC_{0-\infty}$ (ng·hr/mL)	64.2 ± 11.7	18.4 ± 3.5	2.3 ± 0.2	2363 b)
$t_{1/2}$ (hr)	2.0 ± 0.7	1.8 ± 0.8	0.8 ± 0.1	96.7
CLtot (mL/min/kg)	–	–	–	–
Vd (L/kg)	–	–	–	–
BA (%)	–	–	–	–
Study No.	KSI 137/013760			

LSC: Liquid scintillation counter, TRA: Total radioactivity
a) ng Eq/mL.
b) ng Eq·hr/mL.
-: Not calculated. Data represents mean ± S.D.

Total excretion of radioactivity in 7 days following a single oral administration of ^{14}C-KMD-3213 (0.5 mg/kg) to male dogs (results expressed as total % dose)

Sample	Animal no./Sex			
	1M	3M	5M	Mean ± SD
Urine	[redacted]	[redacted]	[redacted]	24.62 ± 9.82
Faeces				61.18 ± 11.06
Cage wash	[redacted]	[redacted]	[redacted]	3.73 ± 0.33
Total	90.61	89.92	88.07	89.53 ± 1.31

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SD Standard deviation

Humans: The CL_{tot} of silodosin was 167 mL/min in humans after continuous intravenous injection for 4 hours. The BA in fasting humans after a single oral administration of silodosin was about 32%. The CL_{tot} of silodosin adjusted with the R_B value was about 20% of the hepatic blood flow in humans. It was determined that the first-pass effect occurring in the gastrointestinal tract, as well as the first-pass effect in the liver, affected the BA of silodosin in humans.

Species	Humans			Humans	Humans	Humans
Sex (M/F)/N=	M/11			M/6	M/9	M/6
Feeding	Fasting	Non-fasting	Fasting	Fasting	Non-fasting	Fasting
Vehicle/dosing form	Capsule	Capsule	(Preparation for injection)	Capsule	Capsule	Solution in dilute hydrochloric acid
Administration method	Oral	Oral	Intravenous drip infusion	p.o.	p.o.	p.o.
Dose (mg)	4	4	2	4	4	8 a)
Sample	Plasma	Plasma	Plasma	Plasma	Plasma	Plasma
Substance to be assayed (target substance)	silodosin	silodosin	silodosin	silodosin	silodosin	TRA
Assay method	HPLC	HPLC	HPLC	HPLC	LC/MS/MS	LSC
PK parameters:						
Tmax (hr)	1.4 ± 1.1	2.1 ± 0.7	4.0	1.4 ± 1.8	2.3 ± 0.5	2.17 ± 1.37
Cmax (ng/mL)	28.0 ± 9.6	23.0 ± 10.8	42.9 ± 7.9	32.1 ± 8.3	20.5 ± 6.5	230 ± 53.2 c)
AUC0-∞ (ng·hr/mL)	133.7 ± 57.8	128.0 ± 65.7	206.9 ± 40.6	112.4 ± 13.9	121.5 ± 38.1	3560 ± 406 d)
t1/2 (hr)	4.7 ± 3.7	6.0 ± 4.8	3.6 ± 1.7	4.7 ± 2.7	8.7 ± 3.1	125 ± 34
CLtot (mL/min)	–	–	167.0 ± 33.8	–	–	–
Vd (L/kg)	–	–	0.8 ± 0.2	–	–	–
BA (%)	32.2 ± 11.3	–	–	–	–	–
Mean body weight (kg)	61.1			60.2	64.0	79.1
Study No.	KMD-308			98363	KMD-105 b)	KMD3213-US012-99

Data represents mean ± S.D. LSC: Liquid scintillation counter, TRA: Total radioactivity, -: Not calculated

a) Dose per kg body weight calculated based on the mean body weight of 6 subjects (79.1 kg): 0.10 mg/kg, b) Results in non-elderly subjects are presented in order for comparison with the data in healthy adult volunteers, c) ng Eq/mL, d) ng Eq·hr/mL

Note: As for concentrations in human plasma, only results of measured concentrations of silodosin are presented. CLtot and Vd presented are only results after intravenous injection. Parameter values obtained in the study on dose of non-labeled compound were rounded off to the nearest tenth.

A study of KMD-3213 metabolites in mice (Slc:ICR) after a single oral administration (10235). After a single oral dose of silodosin in male and female mice at doses of 20, 100 and 500 mg/kg for males and 60, 150 and 400 mg/kg for females, the T_{max} of silodosin in plasma occurred within 2.0 hours in both sexes. The AUC_{0-24} and C_{max} of silodosin and its metabolites increased with increasing doses. The percentage of the AUC_{0-24} of silodosin relative to the total AUC_{0-24} was 50% or more.

KMD-3213	Males (mg/kg)			Females (mg/kg)		
	20	100	500	60	150	400
AUC_{0-24} (ughr/ml)	0.42	6.42	51.68	5.17	12.83	46.81
AUC_{0-24} (%)	85.7	61.8	58.6	59.1	60.9	59.2
Cmax (ug/ml)	0.17	1.55	6.10	1.36	2.92	7.11
Tmax (hr)	1	0.5	2	0.5	0.5	1

KMD-3293	Males (mg/kg)			Females (mg/kg)		
	20	100	500	60	150	400
AUC_{0-24} (ughr/ml)	0.00	0.66	7.19	0.74	1.26	6.70
AUC_{0-24} (%)	0.0	6.4	8.2	8.5	6.0	8.5
Cmax (ug/ml)	0.00	0.24	1.01	0.17	0.40	0.97
Tmax (hr)	-	0.5	2	0.5	0.5	1

KMD-3295	Males (mg/kg)			Females (mg/kg)		
	20	100	500	60	150	400
AUC_{0-24} (ughr/ml)	0.04	1.43	10.54	1.75	2.54	9.60
AUC_{0-24} (%)	8.2	13.8	11.9	20.0	12.0	12.1
Cmax (ug/ml)	0.03	0.31	1.22	0.30	0.59	1.22
Tmax (hr)	1	1	2	0.5	0.5	1

KMD-3241	Males (mg/kg)			Females (mg/kg)		
	20	100	500	60	150	400
AUC_{0-24} (ughr/ml)	0.01	0.87	10.79	0.74	2.68	9.65
AUC_{0-24} (%)	2.0	8.4	12.2	8.5	12.7	12.2
Cmax (ug/ml)	0.01	0.28	1.14	0.28	0.70	1.33
Tmax (hr)	1	1	2	0.5	0.5	1

KMD-3289	Males (mg/kg)			Females (mg/kg)		
	20	100	500	60	150	400
AUC_{0-24} (ughr/ml)	0.02	1.01	8.02	0.35	1.77	6.31
AUC_{0-24} (%)	4.1	9.7	9.1	4.0	8.4	8.0
Cmax (ug/ml)	0.01	0.24	0.65	0.19	0.37	0.73
Tmax (hr)	1	1	2	0.5	0.5	1

A toxicokinetic study of KMD-3213 in rats (Sprague-Dawley) after a single oral administration (10216). After a single oral dose of silodosin in male and female rats at doses of 100, 300 and 600 mg/kg, the plasma concentration of silodosin reached C_{max} within 1.0 and 4.0 hours after administration, respectively. The AUC_{0-24} of silodosin showed an increase in proportion to the increase in the dose. The increase in AUC_{0-24} was slightly smaller in females than in males. The percentage of the AUC_{0-24} of silodosin was not less than 70% of the total AUC_{0-24} of determined substances.

KMD-3213	Males (mg/kg)			Females (mg/kg)		
	100	300	600	100	300	600
AUC_{0-24} (ughr/ml)	5.04	13.09	22.23	4.33	9.65	14.83
AUC_{0-24} (%)	79.7	72.0	71.4	92.5	82.5	79.6
Cmax (ug/ml)	0.86	1.71	1.68	0.93	1.02	1.05
Tmax (hr)	1	0.5	1	0.5	4	1

KMD-3293	Males (mg/kg)			Females (mg/kg)		
	100	300	600	100	300	600
AUC_{0-24} (ughr/ml)	0.05	0.73	0.88	0.01	0.03	0.08
AUC_{0-24} (%)	0.8	4.0	2.8	0.2	0.3	0.4
Cmax (ug/ml)	0.03	0.09	0.13	0.02	0.02	0.06
Tmax (hr)	0.5	0.5	1	0.5	0.5	0.5

KMD- 3295	Males (mg/kg)			Females (mg/kg)		
	100	300	600	100	300	600
AUC_{0-24} (ughr/ml)	0.01	0.03	0.13	0.00	0.00	0.00
AUC_{0-24} (%)	0.2	0.2	0.4	0.0	0.0	0.0
Cmax (ug/ml)	0.01	0.03	0.06	0.00	0.00	0.00
Tmax (hr)	1	1	1	-	-	-

KMD-3241	Males (mg/kg)			Females (mg/kg)		
	100	300	600	100	300	600
AUC_{0-24} (ughr/ml)	1.02	3.09	5.80	0.30	1.37	2.50
AUC_{0-24} (%)	16.1	17.0	18.6	6.4	11.7	13.4
Cmax (ug/ml)	0.19	0.35	0.36	0.10	0.15	0.15
Tmax (hr)	1	1	2	0.10	0.15	0.15

KMD-3289	Males (mg/kg)			Females (mg/kg)		
	100	300	600	100	300	600
AUC_{0-24} (ughr/ml)	0.20	1.24	2.11	0.04	0.65	1.22
AUC_{0-24} (%)	3.2	6.8	6.8	0.9	5.6	6.5
Cmax (ug/ml)	0.05	0.09	0.11	0.05	0.08	0.07
Tmax (hr)	1	1	2	1	4	0.5

A study of KMD-3213 metabolites in dogs (male Beagle) after a single oral administration (10211). After a single oral dose of silodosin in male and female dogs at doses of 100 and 200 mg/kg, the plasma concentration of silodosin reached C_{max} within 2.0 hours. The AUC_{0-24} of silodosin did not show an increase in proportion to the increase in the dose. The percentage of the AUC_{0-24} of silodosin was not less than 50% of the total AUC_{0-24} of determined substances.

KMD-3213	Males (mg/kg)	
	100	200
AUC_{0-24} (ughr/ml)	32.46	39.63
AUC_{0-24} (%)	60.23	53.85
Cmax (ug/ml)	5.47	5.102
Tmax (hr)	2	2

KMD-3293	Males (mg/kg)	
	100	200
AUC_{0-24} (ughr/ml)	5.22	9.56
AUC_{0-24} (%)	9.69	12.99
Cmax (ug/ml)	0.89	1.78
Tmax (hr)	2	2

KMD- 3295	Males (mg/kg)	
	100	200
AUC_{0-24} (ughr/ml)	1.00	1.52
AUC_{0-24} (%)	1.86	2.07
Cmax (ug/ml)	0.20	0.41
Tmax (hr)	2	2

KMD-3241	Males (mg/kg)	
	100	200
AUC_{0-24} (ughr/ml)	10.79	12.90
AUC_{0-24} (%)	20.02	17.53
Cmax (ug/ml)	1.13	1.29
Tmax (hr)	2	2

KMD-3289	Males (mg/kg)	
	100	200
AUC_{0-24} (ughr/ml)	4.42	9.98
AUC_{0-24} (%)	8.20	13.56
Cmax (ug/ml)	0.37	0.83
Tmax (hr)	4	2

Rats: Absorption site of ^{14}C-KMD-3213 in male rat (SD) alimentary tract (PK10149).

The purpose of this study was to estimate absorption of KMD-3213 in alimentary tract of male rats, The radioactivity in plasma was measured after injections of ^{14}C-KMD-3213 (1 mg/kg) into isolated gut loop of stomach and intestinal tract (duodenum, jejunum and ileum) by ligature. The radioactivities of the residual and the tissues in gut loop were measured. The maximum radioactivity concentrations in plasma after the administration to ligated stomach, duodenum, jejunum and ileum were 11.6. $\pm$ 6.8 (at 4 hours after the administration), 251.0 $\pm$ 119.6 (at 1 hour after the administration) 275.3 $\pm$ 65.0 (at 2 hours after administration) and 202.6 $\pm$ 68.7 ng eq. of KMD-3213/mL (at 1 hour after administration), respectively. The radioactivity concentrations in plasma after the injection to ligated duodenum, jejunum and ileum were at the similarly level as each other. In contrast, the radioactivity concentration in plasma after the administration to ligated stomach was at very lower level than the others. The dose recovery at 4 hours after administration to ligated stomach, duodenum, jejunum and ileum were 91.0 $\pm$ 7.4, 16.8 $\pm$ 4.9, 31.1 $\pm$ 14.7, and 24.5 $\pm$ 11.6%, respectively. The results indicated that '°C-KMD-3213 is widely absorbed in the whole area of intestine of rats, but poorly absorbed from the stomach.

Species/strain:	Rats/SD	Sex (M/F)/N=:	M/3
Feeding:	Fasting	Vehicle/dosing form:	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)
Dose (mg/kg):	1	Administration method:	Injection into the ligated gut loop
Radionuclide:	^{14}C	Specific radioactivity:	2.5 MBq/mg
Target substance to be assayed:	TRA	Assay method:	LSC

Study No.: PK10149

Time (hr)	Plasma radioactivity level (ng Eq/mL)			
	Stomach	Duodenum	Jejunum	Ileum
0.083	N.D.	35.9 ± 6.4	57.3 ± 17.4	46.1 ± 18.6
0.25	1.7 ± 1.8	133.3 ± 48.6	109.2 ± 31.8	103.8 ± 33.7
0.5	3.9 ± 1.9	234.4 ± 134.2	167.6 ± 62.0	155.5 ± 38.7
1	5.1 ± 2.5	251.0 ± 119.6	231.0 ± 72.1	202.6 ± 68.7
2	7.9 ± 4.8	248.1 ± 80.3	275.3 ± 65.0	196.0 ± 55.1
4	11.6 ± 6.8	224.3 ± 110.2	184.0 ± 72.3	146.2 ± 41.7
Gastrointestinal residual rate	91.0 ± 7.4	16.8 ± 4.9	31.1 ± 14.7	24.5 ± 11.6

Data represents mean ± S.D.

LSC: Liquid scintillation counter.

TRA: Total radioactivity.

N.D.: Not detected (below the detection limit).

Pharmacokinetics: Absorption after Repeated Doses

Mice: Multiple Dose Test Article: Silodosin

AUC and C_{max} increased in a dose-dependent manner for both silodosin and its metabolites.

Report Title: Pharmacokinetics of KMD 3213 in Mice following 14-Day Administration in the Diet

Species / Strain: Mouse/CD-1 | Duration of Dosing: 14 days | Study No. RTI-989 and 07-8989

Initial Age: 5-7 weeks | Test Article: Silodosin

Date of First Dose: Not provided | Method of Administration: Oral administration via the diet | GLP Compliance: Yes

Vehicle / Formulation: AIN-93M test diet

Special Features: Blood samples were collected at 0, 4, 8, 12, 16, 20 and 24 hours post dose. Samples were analyzed for levels of test article and its metabolites using a validated LC-MS/MS method. The results of the pharmacokinetic analyses of the animal data are presented in the Animal Exposure Comparison report.

Sex	Dose (mg/kg/day)	AUC_{0-t} (ng·hr/mL)	T_{max} (hr)	C_{max} (ng/mL)
		Silodosin		
Male	20	226	13	17
	60	1459	16	157
	100	3284	17	303
Female	60	1957	16	160
	150	10683	15	772
	400	26827	19	1727
		KMD-3293		
Male	20	7	9	2
	60	202	8	20
	100	431	12	67
Female	60	443	11	83
	150	2934	7	761
	400	6126	19	912
		KMD-3241		
Male	20	23	13	2
	60	270	16	30
	100	617	19	54
Female	60	331	16	26
	150	2208	13	168
	400	4763	19	305
		KMD-3289		
Male	20	80	15	6
	60	479	11	43
	100	1225	17	99
Female	60	462	20	31
	150	3421	21	229
	400	9518	12	642
		KMD-3295		
Male	20	70	11	8
	60	464	8	39
	100	723	11	95
Female	60	921	11	105
	150	4020	7	735
	400	4705	16	565

Best Possible Copy

Rats: Multiple Dose Test Article: Silodosin

AUC and C_{max} increased in a dose-dependent manner for both silodosin and its metabolites.

Report Title: Pharmacokinetics of KMD 3213 in Rats Following 14-Day Administration in Diet

Species / Strain: Rats/Sprague Dawley | Duration of Dosing: 14 days | Study No. RTI-988 and 07-8988

Initial Age: 7-12 weeks | Test Article: Silodosin

Date of First Dose: March 14, 2007 | Method of Administration: Oral administration via the diet | GLP Compliance: Yes

Vehicle / Formulation: ——. lab diet

b(4)

Special Features: Blood samples were collected at 0, 4, 8, 12, 16, 20 and 24 hours post dose. Samples were analyzed for the levels of test article and metabolites using a validated LC-MS/MS method. The results of the pharmacokinetic analyses of the animal data are presented in the Animal Exposure Comparison report.

Sex	Dose (mg/kg/day)	AUC_{0-t} (ng•hr/mL)	T_{max} (hr)	C_{max} (ng/mL)
		Silodosin		
Male	15	203	19	13
	50	942	20	81
	150	2947	17	228
Female	15	163	19	13
	80	1194	12	106
	250	3078	24	352
		KMD-3241		
Male	15	34	21	3
	50	407	13	40
	150	1607	23	135
Female	15	38	19	3
	80	463	17	37
	250	993	24	90
		KMD-3289		
Male	15	2	7	0
	50	254	13	28
	150	1417	21	115
Female	15	3	0	0
	80	277	19	24
	250	817	23	76
		KMD-3293		
Male	15	14	4	3
	50	158	13	15
	150	523	5	43
Female	15	0	0	0
	80	105	21	11
	250	315	24	41
		KMD-3310		
Male	15	426	20	40
	50	1912	11	177
	150	5288	20	383
Female	15	279	20	27
	80	1600	17	131
	250	3275	21	262

Best Possible Copy

Dogs: Multiple Dose Test Article: Silodosin

AUC and C_{max} values were higher in males compared to females for both silodosin and its metabolites.

Report Title: Pharmacokinetics of KMD 3213 in Dogs following 14-Day Oral Administration

Species / Strain: Dogs/Beagle Duration of Dosing: 14 days Study No. RTI-987 and 07-8987

Initial Age: Not provided Test Article: Silodosin

Date of First Dose: Not provided Method of Administration: Oral administration (capsules) GLP Compliance: Yes

Vehicle / Formulation: 000 gelatin capsules

Special Features: Blood samples were collected at 0, 4, 8, 12, 16, 20 and 24 hours post dose. Samples were analyzed for levels of test article and metabolites using a validated LC-MS/MS method. The results of the pharmacokinetic analyses of the animal data are presented in the Animal Exposure Comparison report.

Sex	Dose (mg/kg/day)	AUC_{0-t} (ng•hr/mL)	T_{max} (hr)	C_{max} (ng/mL)
		Silodosin		
Female	80	22065	4	3477
Male	80	36977	4	5303
		KMD-3241		
Female	80	7958	4	1023
Male	80	13646	4	1387
		KMD-3289		
Female	80	7535	4	674
Male	80	13869	4	1109
		KMD-3293		
Female	80	711	4	153
Male	80	911	4	178
		KMD-3295		
Female	80	177	4	37
Male	80	384	4	67
		KMD-3310		
Female	80	56760	5	5077
Male	80	74911	4	6947

Humans: Multiple Dose Test Article: Silodosin

In healthy male human volunteers given a single oral administration of 8 mg of ^{14}C-KMD-3213, KMD-3213G and KMD-3293 were mainly detected in plasma, along with silodosin

Report Title: An Investigation of the Exposure of Silodosin and Metabolites KMD-3213G, KMD-3293, KMD-3295, and KMD-3310 after Multiple Doses of Silodosin 8 mg in Healthy Target-Aged Males

Species: Humans | Duration of Dosing: 7 days | Study No. SI06004

Initial Age: Not provided | Test Article: Silodosin

Date of First Dose: July 8, 2006 | Method of Administration: Oral administration (capsules) | GCP Compliance: Yes

Vehicle / Formulation: Silodosin Capsules, 4 mg Clinical formulation used in Phase 3

Special Features: Blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 hours post dose on Day 7. Samples were analyzed for levels of test article and metabolites using a validated LC-MS/MS method. The results of the pharmacokinetic analyses of the animal data are presented in pharmacokinetic reports 06-8911, 06-8912, and 06-8913.

Sex	Dose (mg/day)	AUC_{0-24} (ng•hr/mL)	T_{max} (hr)	C_{max} (ng/mL)
			Silodosin	
Male	8*	373.4	2.6	61.6
			KMD-3213G	
Male	8*	1660.5	5.5	102.4
			KMD-3293	
Male	8*	373.0	4.1	34.3
			KMD-3295	
Male	8*	16.8	4.8	3.4
			KMD-3310	
Male	8*	2.8	3.0	1.6

* Two silodosin 4 mg capsules once daily at breakfast time for 7 days.

2.6.4.4 Distribution

Organ Distribution

Rats: Single Dose Test Article: Silodosin

In fasting rats receiving a single oral administration of 1 mg/kg ^{14}C-KMD-3213, radioactivity was rapidly distributed into the organs and tissues (see Section 2.6.4.4.1). High concentrations of radioactivity were determined 30 minutes after administration in the liver, kidneys, and bladder in addition to the intestinal tract. High concentrations of radioactivity were determined in the liver and kidneys, and comparatively high concentrations were in the pituitary, the pancreas and the bladder 4 hours after administration. At 24 hours post-dose, the highest concentration of radioactivity was determined in the liver and the second highest concentration was in the pituitary. The radioactive concentrations decreased with time in most of the organs and tissues. Higher radioactivity was determined in the liver and the kidneys than in the other organs and tissues 168 hours after administration. Radioactivity disappeared more rapidly from plasma compared to other organs and tissues, and at 168 hours after administration, radioactivity was below the detection level in plasma. In the prostate, concentrations of radioactivity were as high as the concentrations in plasma, with radioactivity being detected even 168 hours after administration. In addition, the elimination half-lives of radioactivity in the testis, brown fat, white fat, skin, and heart were longer than those in other organs and tissues. Throughout all the time points, radioactive concentrations in the cerebrum and the cerebellum were lower than those in plasma. The distribution of silodosin to the central nervous system was low.

Report Title:	Tissue Distribution of Radioactivity in Rats after Single Oral Administration of ^{14}C-KMD-3213		
Species/strain:	Rats/SD	Sex (M/F)/N=:	M/3
Feeding:	Fasting	Vehicle/dosing form:	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)
Dose (mg/kg):	1	Administration method:	Forced oral administration
Radionuclide:.	14C	Specific radioactivity:	2.75 MBq/mg
Target substance to be assayed:	TRA	Assay method:	LSC
Measurement time:	0.5, 4, 24, 72 and 168 hr		
Study No.:	AE-3513-G		

Organs・tissues	Radioactivity concentration (ng Eq/g or mL)									
	0.5 hr		4 hr		24 hr		72 hr		168 hr	$t_{1/2}$
	Concentration	T/P	Concentration	T/P	Concentration	T/P	Concentration	T/P	Concentration	(day)
Plasma	26.4 ± 16.6	(1.00)	45.9 ± 5.2	(1.00)	5.5 ± 1.8	(1.00)	2.3 ± 0.1	(1.00)	N.D.	1.6
Blood	17.7 ± 11.4	(0.67)	33.1 ± 4.5	(0.72)	3.5 ± 0.8	(0.64)	2.5 ± 0.4	(1.09)	N.D.	4.1
Cerebrum	4.3 ± 2.5	(0.16)	10.5 ± 1.3	(0.23)	N.D.	–	N.D.	–	N.D.	-
Cerebellum	4.2 ± 2.2	(0.16)	10.0 ± 1.5	(0.22)	0.9 ± 0.3	(0.16)	0.7 ± 0.3	(0.30)	N.D.	5.5
Spine	3.2 ± 1.3	(0.12)	8.2 ± 1.7	(0.18)	N.D.	–	N.D.	–	N.D.	-
Pituitary	33.0 ± 18.0	(1.25)	288.4 ± 111.7	(6.28)	33.7 ± 36.7	(6.13)	N.D.	–	N.D.	-
Eyeball	6.1 ± 3.7	(0.23)	26.9 ± 7.1	(0.59)	2.8 ± 0.2	(0.51)	0.8 ± 0.4	(0.35)	0.9 ± 0.1	4.4
Harderian gland	20.1 ± 8.2	(0.76)	127.9 ± 37.1	(2.79)	18.5 ± 4.5	(3.36)	3.5 ± 1.0	(1.52)	3.7 ± 1.5	3.0
Thyroid	32.9 ± 14.4	(1.25)	112.6 ± 21.9	(2.45)	N.D.	–	N.D.	–	N.D.	-
Trachea	9.9 ± 6.4	(0.38)	43.3 ± 2.5	(0.94)	N.D.	–	N.D.	–	N.D.	-
Mandibular gland	38.2 ± 18.2	(1.45)	133.8 ± 29.7	(2.92)	7.4 ± 1.8	(1.35)	2.9 ± 0.6	(1.26)	2.3 ± 0.6	4.0
Thymus	9.6 ± 5.6	(0.36)	29.6 ± 5.3	(0.64)	3.8 ± 0.9	(0.69)	2.8 ± 0.5	(1.22)	2.1 ± 0.6	7.3
Heart	42.5 ± 26.4	(1.61)	140.6 ± 25.6	(3.06)	4.1 ± 1.0	(0.75)	2.3 ± 0.6	(1.00)	2.6 ± 0.2	11
Lung	56.0 ± 27.2	(2.12)	197.3 ± 13.1	(4.30)	10.2 ± 1.2	(1.85)	3.8 ± 0.5	(1.65)	2.6 ± 0.2	3.3
Liver	551.8 ± 291.9	(20.90)	1791.6 ± 454.2	(39.03)	108.6 ± 23.2	(19.75)	47.8 ± 9.3	(20.78)	33.9 ± 8.2	3.9
Kidney	193.7 ± 128.0	(7.34)	463.4 ± 37.3	(10.10)	14.3 ± 5.8	(2.60)	7.1 ± 0.1	(3.09)	6.2 ± 0.8	5.6
Adrenal	43.7 ± 26.9	(1.66)	151.6 ± 39.2	(3.30)	6.4 ± 2.3	(1.16)	N.D.	–	N.D.	-
Spleen	37.8 ± 23.5	(1.43)	113.3 ± 13.7	(2.47)	9.8 ± 7.4	(1.78)	3.4 ± 0.5	(1.48)	4.1 ± 0.5	5.9
Pancreas	55.2 ± 33.0	(2.09)	332.7 ± 140.8	(7.25)	5.3 ± 1.0	(0.96)	2.9 ± 0.5	(1.26)	2.7 ± 0.3	7.0
White fat	6.8 ± 5.0	(0.26)	24.9 ± 9.7	(0.54)	1.8 ± 0.3	(0.33)	1.6 ± 0.5	(0.70)	1.4 ± 0.2	17
Brown fat	27.9 ± 16.5	(1.06)	175.0 ± 84.8	(3.81)	6.6 ± 1.1	(1.20)	4.4 ± 0.6	(1.91)	4.9 ± 1.3	18
Skeletal muscle	12.2 ± 7.7	(0.46)	44.5 ± 10.1	(0.97)	1.8 ± 0.3	(0.33)	1.1 ± 0.3	(0.48)	1.8 ± 0.4	-
Skin	11.2 ± 6.4	(0.42)	37.1 ± 7.8	(0.81)	5.4 ± 0.2	(0.98)	3.0 ± 0.3	(1.30)	3.6 ± 0.6	13
Bone marrow	21.6 ± 14.1	(0.82)	83.5 ± 18.3	(1.82)	9.1 ± 2.8	(1.65)	4.2 ± 0.6	(1.83)	N.D.	1.8
Artery	112.2 ± 67.8	(4.25)	42.3 ± 3.6	(0.92)	N.D.	–	3.3 ± 1.4	(1.43)	3.9 ± 0.7	-
Lymph node	44.8 ± 21.5	(1.70)	132.6 ± 40.7	(2.89)	4.9 ± 1.4	(0.89)	2.5 ± 0.7	(1.09)	2.1 ± 0.7	5.4
Testis	2.9 ± 1.8	(0.11)	11.9 ± 3.3	(0.26)	4.7 ± 0.4	(0.85)	3.1 ± 0.5	(1.35)	3.7 ± 0.4	25
Epididymis	7.0 ± 4.6	(0.27)	24.2 ± 5.9	(0.53)	6.2 ± 1.1	(1.13)	2.3 ± 0.4	(1.00)	1.9 ± 0.4	3.9
Prostate	27.1 ± 20.4	(1.03)	49.6 ± 12.9	(1.08)	5.0 ± 1.5	(0.91)	2.5 ± 0.5	(1.09)	1.4 ± 0.2	3.4
Deferent duct	10.0 ± 6.4	(0.38)	32.7 ± 12.4	(0.71)	7.3 ± 0.3	(1.33)	3.6 ± 0.2	(1.57)	2.6 ± 0.8	4.4
Bladder	143.6 ± 49.6	(5.44)	357.3 ± 34.6	(7.78)	7.5 ± 3.4	(1.36)	N.D.	–	N.D.	-
Stomach	2326.1 ± 1601.8	(88.11)	278.0 ± 94.0	(6.06)	6.4 ± 1.2	(1.16)	4.3 ± 0.2	(1.87)	2.9 ± 0.3	5.5
Small intestine	6427.9 ± 3310.2	(243.48)	4971.9 ± 4051.5	(108.32)	24.3 ± 8.1	(4.42)	4.8 ± 1.2	(2.09)	2.0 ± 0.7	1.8
Large intestine	32.6 ± 11.3	(1.23)	322.7 ± 160.9	(7.03)	43.0 ± 7.9	(7.82)	3.6 ± 0.8	(1.57)	2.3 ± 0.2	1.6

Data represents mean ± S.D., LSC: Liquid scintillation counter, TRA: Total radioactivity, N.D.: Not detected (below the detection limit),
-: Could not be calculated, T/P: Ratio of tissue/plasma radioactivity concentrations.

Percentage distribution of radioactivity in blood cell after a single oral administration of ^{14}C-KMD-3213 to fasting male rats (1 mg/kg)

time	Hematocrit value (%)	Ratio of radioactivity (%)
30 min.	42	11.0
4 hr	42	19.3
24 hr	41	13.5
72 hr	40	45.1
168 hr	41	NC

Data are expressed as the mean values ± S.D. of three animals.

N.C. : Not calculated

Rats: Repeated Dose Tests Article: Silodosin

In non-fasting rats given repeat oral administrations of ^{14}C-KMD-3213 at 1 mg/kg once daily for 21 days, high concentrations of radioactivity were distributed in the liver, followed by the kidneys, pituitary, and skin.

Report Title:	Tissue Distribution of Radioactivity During and After Repeated Administration of ^{14}C-KMD-3213 in Male Rats		
Species/strain:	Rats/SD	Sex (M/F)/N=:	M/3
Feeding:	Non-fasting	Vehicle/dosing form:	0.5% Aqueous solution of methylcellulose (dissolved in dilute hydrochloric acid)
Dose (mg/kg/day):	1	Administration method:	Forced oral
Radionuclide:	14C	Specific radioactivity:	1.35 MBq/mg
Target substance to be assayed:	TRA	Assay method:	LSC
Measurement time:	Days 1, 3, 7, 14 and 21 - 3 and 7 days after 21-time doses (Days 24 and 28, respectively)		
Study No.:	KMD-PKM501		

Organs and tissues	Radioactivity concentration (ng Eq/g or mL)							
	Day 1		Day 3		Day 7		Day 14	
	Concentration	T/P	Concentration	T/P	Concentration	T/P	Concentration	T/P
Plasma	16.4 ± 3.7	(1.00)	23.2 ± 5.1	(1.00)	29.4 ± 2.5	(1.00)	25.1 ± 3.7	(1.00)
Blood	13.1 ± 2.3	(0.80)	20.6 ± 5.5	(0.89)	32.3 ± 2.4	(1.10)	28.8 ± 5.1	(1.15)
Cerebrum	1.2 ± 1.3	(0.08)	2.2 ± 0.7	(0.10)	3.7 ± 0.7	(0.13)	7.6 ± 1.8	(0.30)
Cerebellum	1.0 ± 0.9	(0.06)	3.3 ± 1.3	(0.14)	5.2 ± 1.7	(0.18)	9.4 ± 1.6	(0.38)
Spinal cord	N.D.	–	3.9 ± 1.1	(0.17)	5.9 ± 1.3	(0.20)	7.9 ± 1.3	(0.32)
Pituitary	N.D.	–	48.9 ± 32.5	(2.11)	46.4 ± 13.4	(1.58)	68.2 ± 59.1	(2.72)
Eyeball	1.8 ± 0.7	(0.11)	4.7 ± 0.7	(0.20)	6.6 ± 1.2	(0.23)	14.1 ± 2.2	(0.56)
Thyroid	16.6 ± 18.2	(1.02)	N.D.	–	19.0 ± 17.1	(0.65)	22.3 ± 3.6	(0.89)
Trachea	N.D.	–	11.2 ± 0.4	(0.48)	21.0 ± 2.0	(0.71)	31.8 ± 4.4	(1.27)
Submaxillary gland	6.8 ± 0.5	(0.41)	12.9 ± 3.4	(0.56)	18.8 ± 1.6	(0.64)	38.9 ± 0.8	(1.55)
Thymus	5.4 ± 2.0	(0.33)	35.0 ± 37.8	(1.51)	18.0 ± 1.3	(0.61)	39.1 ± 2.5	(1.56)
Heart	5.2 ± 1.3	(0.32)	9.6 ± 1.8	(0.42)	15.3 ± 0.6	(0.52)	32.7 ± 3.9	(1.31)
Lung	13.5 ± 0.9	(0.82)	21.6 ± 6.0	(0.93)	26.7 ± 2.2	(0.91)	52.7 ± 5.2	(2.10)
Liver	111.5 ± 14.3	(6.81)	167.1 ± 37.6	(7.21)	229.9 ± 23.2	(7.82)	424.0 ± 156.0	(16.91)
Kidney	17.2 ± 3.1	(1.05)	29.8 ± 3.4	(1.29)	47.2 ± 9.9	(1.61)	100.5 ± 6.6	(4.01)
Adrenal	14.0 ± 4.6	(0.85)	18.4 ± 10.0	(0.79)	25.1 ± 3.7	(0.85)	45.6 ± 0.3	(1.82)
Spleen	9.1 ± 2.6	(0.56)	13.8 ± 3.9	(0.60)	21.1 ± 2.2	(0.72)	39.8 ± 7.1	(1.59)
Pancreas	9.6 ± 2.4	(0.58)	13.0 ± 3.6	(0.56)	21.8 ± 1.2	(0.74)	41.8 ± 3.3	(1.67)
White fat	4.2 ± 3.7	(0.26)	5.3 ± 1.5	(0.23)	10.7 ± 5.2	(0.36)	31.6 ± 1.3	(1.26)
Brown fat	10.1 ± 2.8	(0.62)	12.4 ± 2.0	(0.54)	23.9 ± 2.9	(0.81)	48.9 ± 7.0	(1.95)
Skeletal muscle	2.9 ± 0.6	(0.18)	5.8 ± 0.9	(0.25)	11.9 ± 0.5	(0.40)	24.6 ± 1.8	(0.98)
Skin	6.8 ± 1.6	(0.41)	14.7 ± 2.6	(0.63)	27.4 ± 8.9	(0.93)	63.5 ± 10.9	(2.53)
Bone marrow	10.3 ± 8.6	(0.63)	22.6 ± 11.0	(0.98)	25.8 ± 2.4	(0.88)	43.3 ± 6.9	(1.73)
Bone	9.1 ± 3.5	(0.56)	6.1 ± 5.6	(0.26)	13.7 ± 2.4	(0.47)	55.2 ± 48.6	(2.20)
Lymph node	N.D.	–	10.4 ± 1.1	(0.45)	13.5 ± 0.9	(0.46)	39.6 ± 6.5	(1.58)
Testis	6.1 ± 2.1	(0.37)	14.3 ± 1.2	(0.62)	27.0 ± 3.5	(0.92)	46.1 ± 2.0	(1.84)
Epididymis	5.1 ± 0.7	(0.31)	9.9 ± 0.7	(0.43)	13.0 ± 1.6	(0.44)	26.3 ± 1.6	(1.05)
Prostate	6.2 ± 1.3	(0.38)	10.7 ± 1.9	(0.46)	14.9 ± 3.4	(0.51)	32.7 ± 6.6	(1.30)
Vas deferens	7.4 ± 1.2	(0.45)	15.6 ± 3.4	(0.67)	19.5 ± 0.6	(0.66)	32.4 ± 3.2	(1.29)
Bladder	15.5 ± 13.8	(0.95)	15.3 ± 4.8	(0.66)	21.8 ± 3.2	(0.74)	58.9 ± 26.3	(2.35)

Organs and Tissues	Radioactivity concentration (ngEq/g or mL)					
	Day 21		Day 24		Day 28	
	Concentration	T/P	Concentration	T/P	Concentration	T/P
Plasma	22.8 ± 6.1	(1.00)	12.5 ± 4.2	(1.00)	5.9 ± 0.3	(1.00)
Blood	36.2 ± 4.5	(1.59)	26.4 ± 5.8	(2.10)	26.6 ± 4.3	(4.55)
Cerebrum	10.0 ± 0.8	(0.44)	10.1 ± 1.9	(0.81)	8.5 ± 1.0	(1.45)
Cerebellum	13.3 ± 2.8	(0.58)	12.9 ± 2.6	(1.03)	11.8 ± 1.9	(2.01)
Spinal cord	7.7 ± 5.0	(0.34)	8.4 ± 1.3	(0.67)	6.7 ± 4.8	(1.14)
Pituitary	87.1 ± 27.8	(3.81)	36.7 ± 32.5	(2.93)	N.D.	–
Eyeball	17.8 ± 1.0	(0.78)	15.2 ± 2.9	(1.21)	11.4 ± 1.8	(1.94)
Thyroid	52.2 ± 19.9	(2.28)	40.3 ± 16.1	(3.21)	30.5 ± 7.3	(5.20)
Trachea	38.5 ± 6.7	(1.68)	36.1 ± 14.0	(2.88)	30.1 ± 6.9	(5.14)
Submaxillary gland	43.6 ± 5.3	(1.91)	33.8 ± 7.9	(2.70)	22.0 ± 3.7	(3.75)
Thymus	41.8 ± 6.8	(1.83)	29.0 ± 4.6	(2.31)	16.9 ± 2.1	(2.89)
Heart	42.2 ± 4.2	(1.85)	33.6 ± 5.1	(2.68)	29.9 ± 2.3	(5.10)
Lung	54.9 ± 6.5	(2.41)	40.0 ± 9.4	(3.19)	30.5 ± 3.1	(5.20)
Liver	637.5 ± 117.8	(27.92)	282.9 ± 64.4	(22.57)	223.7 ± 117.9	(38.20)
Kidney	125.5 ± 6.6	(5.50)	93.5 ± 4.9	(7.46)	74.3 ± 12.1	(12.69)
Adrenal	48.5 ± 3.1	(2.12)	42.5 ± 14.8	(3.39)	33.9 ± 7.3	(5.78)
Spleen	49.1 ± 1.8	(2.15)	36.3 ± 7.4	(2.90)	25.4 ± 2.3	(4.34)
Pancreas	45.8 ± 1.4	(2.01)	31.2 ± 6.0	(2.49)	26.4 ± 2.7	(4.51)
White fat	25.9 ± 4.2	(1.13)	15.8 ± 7.9	(1.26)	16.9 ± 4.0	(2.89)
Brown fat	47.3 ± 19.0	(2.07)	36.2 ± 8.6	(2.89)	38.1 ± 3.8	(6.51)
Skeletal muscle	30.9 ± 5.9	(1.35)	26.6 ± 5.9	(2.12)	23.2 ± 9.3	(3.96)
Skin	81.6 ± 15.6	(3.57)	72.7 ± 15.6	(5.80)	27.6 ± 12.6	(4.71)
Bone marrow	51.8 ± 7.1	(2.27)	33.1 ± 7.2	(2.64)	19.8 ± 4.3	(3.38)
Bone	43.4 ± 8.2	(1.90)	27.5 ± 12.6	(2.19)	12.5 ± 1.3	(2.13)
Lymph node	39.9 ± 5.6	(1.75)	28.4 ± 6.2	(2.27)	19.6 ± 9.7	(3.35)
Testis	60.4 ± 8.5	(2.65)	55.8 ± 5.3	(4.45)	31.8 ± 10.6	(5.42)
Epididymis	34.6 ± 3.6	(1.51)	26.0±3.9	(2.08)	30.0 ± 9.6	(5.12)
Prostate	30.0 ± 2.6	(1.32)	22.4 ± 4.6	(1.79)	12.9 ± 0.6	(2.20)
Vas deferens	38.0 ± 0.2	(1.66)	33.1 ± 6.8	(2.64)	15.4 ± 12.3	(2.63)
Bladder	49.5 ± 2.8	(2.17)	41.5 ± 5.8	(3.31)	17.7 ± 2.5	(3.01)

Data represents mean ± S.D., T/P: Ratio of tissue/plasma radioactivity concentrations.
Note: Concentrations in the gastrointestinal tract are not given in the table since they were measured including the content.

Pharmacokinetics: Plasma Protein Binding Test Article: Silodosin
The binding ratio of silodosin to plasma proteins was highest in humans at 94.6 to 95.8%, whereas it was 80% in rats and dogs. The binding ratios of the glucuronide conjugate of silodosin (KMD-3213G) and KMD-3293, the major metabolites in humans, were 91.2 to 92.0% and 90.2 to 91.9%, respectively. α1-Acid glycoprotein was considered to be the major binding protein for KMD-3293, MD127 and for KMD-3213.

Species	Sample	Substance to be measured	Test concentration (ng/mL)	Binding rate (%)	Study No.
Rats	Plasma	^{14}C-KMD-3213	100 - 500	79.9 - 81.4	PK10153
Dogs	Plasma	^{14}C-KMD-3213	100 - 500	80.1 - 81.6	
Humans	Plasma	^{14}C-KMD-3213	100 - 500	94.6 - 95.8	
Humans	Albumin	^{14}C-KMD-3213	100 - 500	34.7 - 35.4	
Humans	γ-Globulin	^{14}C-KMD-3213	100 - 500	4.6 - 7.4	
Humans	α_1-Acid glycoprotein	^{14}C-KMD-3213	100 - 500	94.3 - 95.0	
Rats	Plasma	KMD-3213G [a)]	200, 500	71.5, 67.5	DMPK2003-0053
Dogs	Plasma	KMD-3213G [a)]	200, 500	59.5, 63.8	
Humans	Plasma	KMD-3213G [a)]	200, 500	92.0, 91.2	
Humans	Albumin	KMD-3213G [a)]	200, 500	33.8, 35.7	
Humans	γ-Globulin	KMD-3213G [a)]	200, 500	20.4, 11.7	
Humans	α_1-Acid glycoprotein	KMD-3213G [a)]	200, 500	84.8, 85.9	
Humans	Plasma	KMD-3293	200, 500	91.9, 90.2	DMPK2004-0033
Humans	Albumin	KMD-3293	200, 500	28.8, 31.1	
Humans	γ-Globulin	KMD-3293	200, 500	7.2, 3.9	
Humans	α_1-Acid glycoprotein	KMD-3293	200, 500	92.1, 92.1	

Test system and methods: *In vitro* test (ultrafiltration method).
Measurement methods: TRA - total radioactivity (LSC - liquid scintillation counter) and fluorescence HPLC.
Data represents mean of 3 cases.
a) Glucuronide conjugate of silodosin.

Pharmacokinetics: Other Distribution Studies

Distribution in Blood Cells Test Article: Silodosin
The blood cell association of silodosin was about 30 to 60% of the administered dose in rat and dog blood, whereas it was not greater than 5% in humans.

Test system: *In vitro* test
Measurement system and method: TRA - total radioactivity (LSC - liquid scintillation counter)

Species	Substance to be Measured	Test concentration (ng/mL)	Transition rate (%)	R_B	Study No.
Rats	^{14}C-KMD-3213	20	49.3	1.11	PK10151
		100	49.7	1.11	
		200	48.1	1.07	
Dogs	^{14}C-KMD-3213	10	29.5, 57.0	0.865, 1.10	KSI 137/013760
		40	31.5, 58.0	0.910, 1.15	
		100	29.5, 57.5	0.865, 1.10	
Humans	^{14}C-KMD-3213	24	2.2	0.51	PK10091
		121	3.7	0.55	

Results in rats and humans present mean of 3 cases, while those in dogs present mean of 2 cases.

A four week oral capsule study in Beagle dogs to investigate the extent of liver and kidney accumulation and pharmacokinetics of KMD-3213 and metabolites KMD-3241 and KMD-3289 following repeat doses of KMD-3213 at 25 mg/kg/day (KSI 115/994798, September, 2000, GLP)

Blood samples were taken on Days 1, 14, 21 and 28 following daily oral administration of KMD-3213 to male dogs at a dose level of 25 mg/kg/day for 4 weeks. Plasma concentrations of KMD-3213, and the metabolites KMD-3241 and KMD-3289, in samples taken up to 24 hours post-dose were measured by a validated high performance liquid chromatographic (HPLC) method. Samples of liver and kidney tissue were taken from animals on Days 1, 14, 21 and 28 after the 24 hour blood samples had been taken,

and the concentrations of KMD-3213, KMD-3241 and KMD-3289 were also measured by validated HPLC methods.

The mean C_{max} and AUC_{24} values for KMD-3213, KMD-3241 and KMD-3289 in plasma are summarized below with standard deviations in parentheses:

	C_{max} (ng/ml)				AUC_{24} (ng.h/ml)			
Analyte	Day 1	Day 14	Day 21	Day 28	Day 1	Day 14	Day 21	Day 28
KMD-3213	1480.70 (663.94)	2702.53 (911.94)	2698.53 (1116.58)	2282.19 (785.90)	6087 (1953)	11069 (2872)	8957 (3114)	11417 (5897)
KMD-3241	467.24 (217.15)	528.67 (155.76)	668.09 (241.47)	529.69 (124.59)	1907 (733)	2969 (1003)	3326 (1320)	3527 (1935)
KMD-3289	71.28 (32.10)	336.37 (140.53)	402.30 (199.77)	308.70 (75.83)	365 (148)	1808 (545)	2126 (949)	2075 (950)

The terminal half-lives of KMD-3213, KMD-3241 and KMD-3289 on Days 1, 14, 21 and 28 were similar, and in the range 1.3 to 4.9 hours.

The mean metabolite ratios for KMD-3241 and KMD-3289 are summarized below:

	KMD-3241	KMD-3289
Day 1	0.308	0.066
Day 14	0.262	0.183
Day 21	0.367	0.263
Day 28	0.306	0.214

There was little or no evidence of accumulation of KMD-3213 or KMD-3241 in liver and kidney tissue (accumulation ratios <2), however, KMD-3289 concentrations in liver and kidney were higher after repeated oral doses of KMD-3213 than after a single dose, and were similar on Days 21 and 28 indicating that steady-state had been attained by Day 21.

The determination by whole body autoradiography of the qualitative tissue distribution of radioactivity in male and pregnant female rats after a single oral administration.

The qualitative tissue distribution of radioactivity was investigated in male and pregnant female rats (Sprague-Dawley CD strain) by whole-body autoradiography, following a single oral administration of ^{14}C-KMD-3213 at a dose level of 1 mg/kg.

During the 24 hours after single oral doses of ^{14}C-KMD-3213 to male rats (*viz* at 0.5, 4 or 24 hours post-dose), highest concentrations of radioactivity were found in the gastrointestinal tract contents and urinary bladder, while medium levels were generally associated with the liver, pituitary, preputial gland, prostate and urethra. Lowest levels of radioactivity were detected in many other tissues, including the adrenal gland, blood, exorbital and intra-orbital lacrimal glands, Harderian gland, kidney, lung, myocardium, pancreas, salivary gland, spleen, thyroid and uveal tract, but not in the brain, eye or spinal cord.

At 48 and 72 hours post-administration, concentrations of radioactivity in all tissues had declined considerably, such that none contained relatively high levels, and only the gastrointestinal tract, liver, pituitary and preputial gland contained medium levels at the former time and only liver at the latter time. The only other tissue associated with radioactivity (at low levels) at both of these times was the kidney.

During the 24 hours after single oral doses of ^{14}C-KMD-3213 to pregnant female rats (*viz* at 0.5, 4 or 24 hours post-dose), highest concentrations of radioactivity were present in the gastrointestinal tract contents and urinary bladder (as was also the case for male rats). In pregnant female rats, however, medium levels were generally associated with a larger number of tissues, *viz* the exorbital and intra-orbital lacrimal glands, fat, kidney, Harderian gland, liver, pituitary, preputial gland, salivary gland, uterus wall and uveal tract. Lowest levels of radioactivity were detected in the adrenal glands, bone marrow, lung, myocardium, ovary, pancreas, placenta and spleen, but none was detected in the brain, eye, spinal cord or fetuses. By 48 hours after dosing, concentrations of radioactivity in all tissues had declined considerably, such that none contained relatively high levels. Medium levels were present in the gastrointestinal tract, kidney, liver, preputial gland and uterus wall, and low levels were detected in the exorbital and intra-orbital lacrimal glands, fat, Harderian gland, pituitary, placenta, salivary gland, small intestine contents and uveal tract.

In summary, radioactivity was rapidly and widely distributed throughout the animal body, although highest tissue concentrations were largely confined to the gastrointestinal tract and urinary bladder, i.e. the principal excretory organs. Medium levels of radioactivity were generally present in liver and certain glandular tissues, but only low levels were found in the brain and spinal cord of both sexes and in the fetuses of pregnant females, thereby demonstrating that the drug did not readily penetrate the blood-brain barrier or undergo appreciable transplacental transfer in rats.

Qualitative tissue distribution of radioactivity by whole-body autoradiography following a single oral dose of ^{14}C-KMD-3213 (1 mg/kg) to male rats

Time of sacrifice	Level of radioactivity	Tissue/organ
30 minutes	High	Oesophagus, stomach and small intestine contents
	Medium	Liver; prostate; urinary bladder
	Low	Adrenal gland; blood; kidney; lung; myocardium; pituitary; salivary gland; spleen; urethra
4 hours	High	Oesophagus, stomach, small intestine, caecum and large intestine contents; urinary bladder
	Medium	Liver; pituitary; urethra
	Low	Adrenal gland; exorbital and intra-orbital lacrimal glands; Harderian gland; kidney; lung; myocardium; pancreas; pituitary; preputial gland; prostate; salivary gland; spleen; thyroid; uveal tract
24 hours	High	Caecum contents
	Medium	Stomach mucosa; large and small intestine contents; liver; pituitary; preputial gland
	Low	Exorbital and intra-orbital lacrimal glands; Harderian gland; kidney; salivary gland; urinary bladder; uveal tract
48 hours	High	None
	Medium	Caecum and large intestine contents; stomach mucosa; liver; pituitary; preputial gland
	Low	Kidney; small intestine contents
72 hours	High	None
	Medium	Liver
	Low	Kidney; large intestine contents; pituitary; preputial gland; stomach mucosa

Qualitative tissue distribution of radioactivity by whole-body autoradiography following a single oral dose of ^{14}C-KMD-3213 (1 mg/kg) to pregnant rats

Time of sacrifice	Level of radioactivity	Tissue/organ
30 minutes	High	Stomach and small intestine contents; oesophagus mucosa
	Medium	Liver; urethra; urinary bladder
	Low	Adrenal gland; bone marrow; exorbital and intra-orbital lacrimal glands; fat; Harderian gland; kidney; large intestine contents; lung; myocardium; ovary; pancreas; pituitary; preputial gland; placenta; salivary gland; spleen; uveal tract
4 hours	High	Oesophagus, stomach and small intestine contents; urinary bladder
	Medium	Exorbital and intra-orbital lacrimal glands; fat; kidney; Harderian gland; liver; pituitary; preputial gland; salivary gland; uterus wall; uveal tract
	Low	Adrenal gland; bone marrow; lung; large intestine contents; myocardium; ovary; pancreas; placenta; spleen
24 hours	High	Caecum contents
	Medium	Stomach mucosa; large intestine and small intestine contents; exorbital and intra-orbital lacrimal glands; Harderian gland; liver; preputial gland; uterus wall; uveal tract
	Low	Fat; kidney; lung; myocardium; ovary; pituitary; placenta; salivary gland; spleen; urinary bladder
48 hours	High	None
	Medium	Stomach and large intestine contents; kidney; liver; preputial gland; uterus wall
	Low	Exorbital and intra-orbital lacrimal glands; fat; Harderian gland; pituitary; placenta; salivary gland; small intestine contents; uveal tract

2.6.4.5 Metabolism

Pharmacokinetics: Metabolism *In Vivo* Test Article: Silodosin & Metabolites

In vitro metabolism studies using rat, dog, and human hepatocytes showed that a glucuronide conjugate of silodosin (KMD-3213G) and a metabolite in which the alcoholic hydroxy group was oxidized (KMD-3293) were mainly synthesized in hepatocytes and are the major metabolites in humans. KMD-3293 was also synthesized in rat and dog hepatocytes, but KMD-3213G was not seen in rat or dog hepatocytes.

Species/strain	Rats/SD	Dogs/Beagle	Humans
Sex (M/F)/N=:	M/3	M/3	M/6
Feeding:	Fasting	Fasting	Fasting
Vehicle/dosing form:	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)	Capsule (dissolved in dilute hydrochloric acid)	Dissolved in dilute hydrochloric acid
Administration method:	Forced oral	Oral	Oral
Dose (mg/kg):	1	0.5	8 mg/body
Radionuclide:	^{14}C	^{14}C	^{14}C
Specific radioactivity:	2.49 MBq/mg	1.46 MBq/mg	0.46 MBq/mg

Species	Sample	Sample time or period	Metabolite in sample (%)										Study No.
			KMD-3213	KMD-3241	KMD-3250	KMD-3289	KMD-3293	KMD-3295	KMD-3299	KMD-3310	KMD-3213G [c]	KMD-3241G [d]	
Rats	Plasma	0.5	4.8	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	74.7	N.D.	–	PK10055
		4	11.9	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	39.9	N.D.	–	
	Urine	0 - 24	12.8	9.7	N.D.	1.3	N.D.	1.0	N.D.	37.9	N.D.	–	
			(2.6)	(2.0)	(N.D.)	(0.3)	(N.D.)	(0.2)	(N.D.)	(7.8)	(N.D.)	–	
	Feces	0 - 24	12.7	9.2	31.6	N.D.	N.D.	9.1	9.5	N.D.	N.D.	–	
			(8.6)	(6.3)	(21.5)	(N.D.)	(N.D.)	(6.2)	(6.5)	(N.D.)	(N.D.)	–	
	Bile	0 - 24	2.3	6.6	25.9	0.8	2.8	16.1	3.6	1.9	N.D.	–	
			(1.6)	(4.6)	(18.2)	(0.6)	(2.0)	(11.3)	(2.5)	(1.3)	(N.D.)	–	
	Liver	0.5	1.4	1.4	25.2	N.D.	N.D.	2.9	5.9	21.6	5.7	–	
		4	4.0	3.8	40.4	2.5	2.1	6.2	6.0	7.3	6.9	–	

Species	Sample	Sample time or period	Metabolite in sample (%)										Study No.
			KMD-3213	KMD-3241	KMD-3250	KMD-3289	KMD-3293	KMD-3295	KMD-3299	KMD-3310	KMD-3213G [c]	KMD-3241G [d]	
Rats	Kidney	0.5	3.8	0.9	N.D.	0.6	N.D.	N.D.	N.D.	69.3	N.D.	–	PK10055
		4	12.7	10.7	N.D.	N.D.	N.D.	N.D.	N.D.	51.4	N.D.	–	
	Prostate	0.5	26.7	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	53.6	N.D.	–	
		4	32.3	18.5	N.D.	N.D.	N.D.	N.D.	N.D.	30.7	N.D.	–	
Dogs	Plasma	0.25	18.1	3.3	–	N.D.	N.D.	N.D.	–	73.1	N.D.	–	KSI 137/013760
		0.5	13.4	1.1	–	N.D.	N.D.	N.D.	–	81.9	N.D.	–	
		1	10.1	1.6	–	N.D.	0.5	0.3	–	84.1	N.D.	–	
		1.5	6.9	1.5	–	N.D.	0.5	0.3	–	84.4	N.D	–	
		2	1.6	0.9	–	N.D.	N.D.	N.D.	–	93.8	N.D	–	
		3	1.6	0.4	–	N.D.	N.D.	N.D.	–	94.9	N.D.	–	
		4	0.5	N.D.	–	N.D.	N.D.	N.D.	–	95.3	N.D.	–	
		6	5.1	2.3	–	N.D.	N.D.	N.D.	–	79.8	N.D.	–	
		8	4.5	2.8	–	N.D.	N.D.	N.D.	–	69.3	N.D.	–	
		12	2.6	N.D.	–	N.D.	N.D.	N.D.	–	60.6	N.D.	–	
		24	N.D.	N.D.	–	N.D.	N.D.	N.D.	–	52.7	N.D.	–	
	Urine	0 - 24	2.1	N.D.	–	N.D.	0.5	0.4	–	81.5	N.D.	–	
			(0.38)	(N.D.)	(–)	(N.D.)	(0.10)	(0.06)	(–)	(18.61)	(N.D.)	–	
	Feces	0 - 24	19.0	5.4	–	N.D.	29.1	24.3	–	1.4	5.7	–	
			(8.71)	(2.28)	(–)	(N.D.)	(12.80)	(10.12)	(–)	(0.62)	(2.36)	–	
		24 - 48	6.5	1.4	–	N.D.	35.1	23.5	–	2.7	5.6	–	
			(1.34)	(0.23)	(–)	(N.D.)	(5.38)	(3.89)	(–)	(0.29)	(0.63)	–	

Species	Sample	Sample time or period	Metabolite in sample (%)										Study No.
			KMD-3213	KMD-3241	KMD-3250	KMD-3289	KMD-3293	KMD-3295	KMD-3299	KMD-3310	KMD-3213G [c]	KMD-3241G [d]	
Humans	Plasma	0.25	68.07	5.39	N.D.	0.79	7.00 [b]	N.D.	N.D.	7.31	0.70	N.D.	KMD-3213-US012-99
		0.5	69.54	4.78	N.D.	N.D.	11.04 [b]	N.D.	N.D.	12.49	0.49	N.D.	
		1	41.89	2.79	N.D.	1.33	23.10 [b]	2.59	N.D.	9.13	9.90	1.67	
		1.5	32.93	3.99	N.D.	1.25	29.18 [b]	4.06	N.D.	6.89	12.45	2.67	
		2	31.26	3.26	N.D.	0.82	37.22 [b]	2.43	N.D.	6.55	13.43	3.12	
		3	40.33	4.14	N.D.	0.90	29.65 [b]	2.39	N.D.	3.81	14.14	1.69	
		4	25.22	2.11	N.D.	0.64	32.98 [b]	3.76	N.D.	3.79	22.64	3.74	
		6	16.58	1.93	N.D.	0.83	41.26 [b]	3.38	N.D.	2.18	21.65	6.09	
		8	10.26	0.67	N.D.	0.70	41.00 [b]	6.15	N.D.	1.05	29.55	6.17	
		10	11.14	1.57	N.D.	N.D.	39.95 [b]	3.54	N.D.	1.41	32.89	6.38	
		12	4.93	0.73	N.D.	N.D.	35.79 [b]	3.53	N.D.	1.66	43.90	4.47	
	Urine	0 – 48	10.77	1.54	N.D.	0.60	20.56 [b]	2.40	N.D.	18.92	7.38	2.11	
			(3.55)	(0.53)	(N.D.)	(0.21)	(6.74 [b])	(0.81)	(N.D.)	(6.50)	(2.42)	(0.70)	
	Feces	0 - r [a]	28.00	10.80	N.D.	1.99	40.26 [b]	9.70	N.D.	N.D.	N.D.	N.D.	
			(15.39)	(5.82)	(N.D.)	(1.06)	(22.30 [b])	(5.29)	(N.D.)	(N.D.)	(N.D.)	(N.D.)	

a) Results obtained from feces collected till the time point when than 94% or more of radioactivity administered was recovered, b) Including [redacted] of KMD-3293 (which was generated from KMD-3293 in the process of extraction), c) Glucuronide conjugate of silodosin, d) Glucuronide conjugate of KMD-3241.

Figures in parenthesis present the results of conversion from the percentage of a metabolite in the sample into the excretion rate.

-: Not measured, N.D.: Not detected (below the detection limit).

Note: Substances were identified by comparing the retention time of synthesized metabolites on HPLC. The percentages of unknown metabolites other than those used in measurement are not given in the above table.

b(4)

Pharmacokinetics: Metabolism In Vitro Test Article: Silodosin

The major human glucuronidated metabolite KMD-3213 was minimally produced in monkey hepatocytes.

Test system: Hepatocytes (frozen hepatocytes)				
Study No.	PK10104			PK-03-519
Species:	Rats	Dogs	Humans	Monkeys [c]
Reaction time:	37 °C, 24 hr	37 °C, 24 hr	37 °C, 24 hr	37 °C, 24 hr
Treatment concentration:	0.1 mmol/L	0.1 mmol/L	0.1 mmol/L	0.1 mmol/L
Nuclide:	^{14}C	^{14}C	^{14}C	^{14}C
Metabolites	Percentage of metabolites (%)			
KMD-3213 (silodosin)	10.8	25.3	0.9	N.D.
KMD-3213G [a] (MD127K)	N.D.	N.D.	35.8	3.7
KMD-3293	49.5 [b]	6.8	42.6	33.7
KMD-3310	6.9	34.1	3.4	26.4
KMD-3241	6.6	14.0	N.D.	N.D.
KMD-3289	1.3	1.9	1.1	N.D.
KMD-3295	8.0	3.6	4.1	19.4
KMD-3250	1.9	0.7	N.D.	0.3
KMD-3299	N.D.	N.D.	N.D.	N.D.
Others	15.1	13.7	12.2	16.5

Data represent mean of 2 cases. N.D.: Not detected (below the detection limit).

a) Glucuronide conjugate of silodosin.

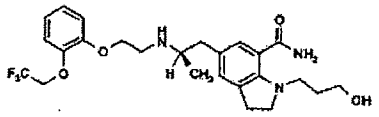
b) Including an unknown metabolite (UK-3).

c) Cynomolgus monkeys.

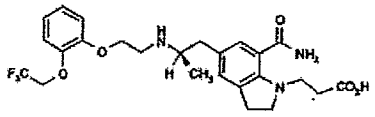
Note: It was confirmed by results of LC/MS that the peak of KMD-3293 in rats included UK-3 (m/z: 576, positive).

See structures below:

APPEARS THIS WAY ON ORIGINAL



KMD-3213



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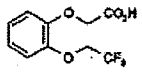
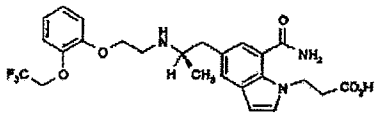
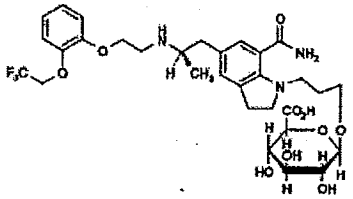


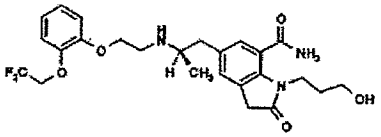
KMD-3310



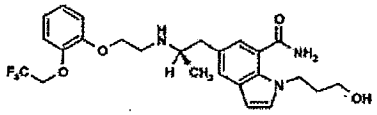
KMD-3295



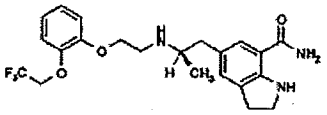
MD127(KMD-3213-glucuronide)



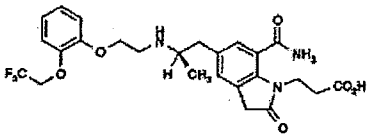
KMD-3250



KMD-3241



KMD-3289



KMD-3299

Pharmacokinetics: Induction / Inhibition of Drug-Metabolizing Enzymes
Rats were administered repeat oral administrations of silodosin at 1, 10, and 30 mg/kg once daily for 7 days and then fasted overnight. The liver was removed from the rats and liver microsomes were prepared from the isolated liver. Drug metabolic enzyme activity was determined using the liver microsomes. The amount of cytochrome P-450 increased in the rats receiving repeat oral administration of 10 and 30 mg/kg daily compared to the negative control group. The aniline 4-hydroxy enzyme activity decreased only in those rats given 10 mg/kg daily compared to rats in the negative control group with 0.83 times the activity of the negative control. The uridine diphosphate glucuronyl transferase (UDP-GT) activity significantly decreased in rats receiving 1 mg/kg daily compared to rats in the negative control group with a 0.78-fold difference. There were no differences in the amount of liver microsome protein, the amount of cytochrome b_5, aminopyrine N-

dimethyl enzyme activity, 7-ethoxy cumarine O-deethylase enzyme activity, or glutathione S-transferase activity between silodosin-treated rats and control rats. From these results, rats receiving a dose that is 100-fold higher than the pharmacological dose (0.1 mg/kg) had a statistically greater increase in the amount of cytochrome P-450 compared to the control group, with an increasing rate of about 20%.

Test Article: Silodosin
Study type: Rat metabolizing enzyme induction test
Species/strain: Rats/SD, Sex (M/F)/N=: M/8
Feeding: Non-fasting

Drug administered:	silodosin (KMD-3213)	phenobarbital [a] (positive control)
Dose (mg/kg/day):	1, 10, 30	80
Vehicle:	0.5% Aqueous solution of methyl cellulose (suspension)	Dissolved in physiological saline
Administration method:	Forced oral administration	Intraperitoneal injection
Report Title:	KMD-YK-04, Effects of Repeated Oral Administration of KMD-3213 on Hepatic Drug-Metabolizing Enzymes in Male Rats	

Investigational product	Control [a]	Silodosin (KMD-3213)			Phenobarbital [a]
Dose (mg/kg/day)	0	1	10	30	80
Body weight at the start of treatment (g)	214 ± 11	210 ± 11	217 ± 7	214 ± 10	216 ± 13
Body weight (g)	219 ± 13	218 ± 17	230 ± 8	221 ± 12	229 ± 18
Liver weight (g)	8.36 ± 0.54	7.88 ± 0.82	9.06 ± 0.19	8.65 ± 0.86	12.39 ± 1.04###
Liver weight (% of body weight)	3.82 ± 0.2	3.63 ± 0.33	3.95 ± 0.16	3.91 ± 0.31	5.44 ± 0.48###
Microsome protein level					
(mg/g liver)	17.0 ± 1.1	18.7 ± 4.2	16.7 ± 1.2	18.2 ± 2.8	24.5 ± 2.4###
(mg/liver)	142 ± 13	146 ± 24	151 ± 11	157 ± 25	302 ± 27###
Cytochrome level (nmol/mg protein)					
Cytochrome P-450 level	1.12 ± 0.09	1.24 ± 0.13	1.25 ± 0.05*	1.37 ± 0.14***	3.08 ± 0.26###
Cytochrome b5 level	0.49 ± 0.16	0.55 ± 0.15	0.45 ± 0.08	0.38 ± 0.05	0.84 ± 0.39##
Enzyme activity (nmol/min/mg protein)					
Aminopyrine N-demethylase	5.51 ± 0.50	5.88 ± 0.35	5.49 ± 0.73	5.97 ± 0.76	10.41 ± 0.54###
Aniline 4-hydroxylase	0.90 ± 0.13	0.89 ± 0.14	0.75 ± 0.08*	0.77 ± 0.12	1.71 ± 0.16###
7-Ethoxycoumarin O-deethylase	1.89 ± 0.17	2.0 ± 0.25	2.0 ± 0.29	1.96 ± 0.15	2.46 ± 0.10###
UDP-GT	37.5 ± 7.6	29.1 ± 7.2*	36.2 ± 5.6	31.1 ± 5.0	52.9 ± 4.9###
Glutathione S-transferase	172 ± 20	173 ± 22	168 ± 19	173 ± 24	280 ± 19###

Data represents mean±S.D. a) Sodium salt, b) Negative control (treated with 0.5% aqueous solution of methyl cellulose).
Dunnett's multiple comparison test (vs. negative control): *; p<0.05, ***; p<0.001
Student's t test (vs. negative control): ##; p<0.01, ###; p<0.001

Assumption of the enzyme responsible for the metabolism of KMD-3213 to its main metabolite KMD-3293 (PK10126). The rate of KMD-3213 formation was measured using human liver microsomal or S9 fractions in the presence of NADPH, NADP or NAD. An insignificant amount of KMD-3293 was formed using microsomes in the absence or presence of NADPH or S9 in the absence of NAD or NADP. In S9 the rates of formation in the presence of NAD and NADP were 2.5 and 0.2 nmol/30 min/2 mg protein, respectively.

Dependency on KMD-3213 concentration in S9 in the presence of NAD (3 mM)

Concentration of KMD-3213 (uM)	10	50	100
Formation of KMD-3293 (nmol/30 min./2 mg prot.)	0.3	1.3	2.5

Effects of inhibitors on metabolism of KMD-3213 (100 uM) in liver S9 in the presence of NAD (3 mM)(30 min./2 mg protein)(N=2)

Inhibitor concentration (uM)	Remaining activity (%)			
	1000	100	10	1
Pyrazole	18.1	38.7	69.1	-

Ethanol	45.7	60.5	59.8	-
Acetaldehyde	60.4	65.9	71.8	-
Disulfiram	-	8.1	82.3	83.1

Based on the above data, it was proposed that alcohol dehydrogenase and aldehyde dehydrogenase are involved in the metabolism of KMD-3213 to KMD-3293.

Identification of the enzyme responsible for production of metabolite KMD-3310 from KMD-3213 (DMPK2003-0037). The rate of KMD-3310 formation was measured using human liver microsomal, S9 fractions, or cytosol in the presence or absence of β-NADPH or β-NADH.

Cofactor requirements for KMD-3310 production in subcellular fractions (N=2)

Subcellular fractions	V ((pmol/min/mg protein)		
	β-NADPH	β-NADH.	none
S9	13.4	BLQ	BLQ
Microsomes	18.0	0.545	BLQ
Cytosol	0.403	BLQ	BLQ

Effect of inhibitors on KMD-3310 production in human liver microsomes (N=2)

Inhibitor	KMD-3310 (% of control)
1 μM α-Naphthoflavone (CYP1A2)	95.3
0.5 μM Tranylcypromine (CYP2A6)	85.8
20 μM Sulfaphenazole (CYP2C9)	80.6
200 μM S-(+)-Mephenytoin (CYP2C19)	77.5
0.5 μM Quinidine (CYP2D6)	82.2
100 μM 4-Methylpyrazole (CYP2E1)	76.6
2 μM Ketoconazole (CYP3A4)	5.60

It was concluded that CYP3A4 is involved in the metabolism of KMD-3213 to KMD-3310.

***In vitro* metabolism study of ^{14}C-KMD-3213—identification of UGT isoforms using microsomes expressing human UGT and determination of Km and Vmax values (AE-3348).** The glucuronidated metabolite MD127 (KMD-3213G) was formed in the presence of human UGT2B7 microsomes (but not in 1A3, 1A6, 1A9, 1A10, 2B7, or 2B15). The mean percent of peak formation was 1.69%, 5.02%, and 8.49% after incubation for 10, 30, and 60 minutes, respectively. The Km and Vmax for glucuronidation of ^{14}C-KMD-3213 by human UGT2B7 were 401.0 umol/L and 670.7 pmol/min/mg protein.

Metabolic profiling in rat plasma, urine, bile and main tissues after a single oral dose of ^{14}C-KMD-3213 (PK10055)

Radiolabeled compound was used to determine the percentages of unchanged drug and metabolites in the plasma, liver, kidneys, prostate, bile, urine and feces by RI-HPLC. KMD-3250 was primarily detected in the liver, feces and bile, and KMD- 3310 in the kidneys, prostate,

plasma and urine of male SD rats. The percentages of unchanged drug at 0.5 and 4 hr after administration were 4.8 and 11.9%, respectively in the plasma, 1.4 and 4.0%, respectively in the liver, 3.8 and 12.7%, respectively in the kidneys, and 26.7 and 32.3%, respectively in the prostate. The percentages of unchanged drug in the urine, feces and bile by 24 hr after administration were 12.8, 12.7 and 2.3%, respectively. In addition, the metabolic profile in the plasma, liver, kidneys and prostate was specific to each organ, showing no marked change over time. Radioactivity concentrations of unchanged drug and metabolites in tissues and their excretion rates after a single oral dose of ^{14}C-KMD-3213 to rats at a dose of 1 mg/kg.

ng eq. KMD-3213/g or mL

Tissue	Sampling time	Total radioactivity concentration	KMD-3213	KMD-3241	KMD-3250	KMD-3289	KMD-3293	KMD-3295	KMD-3299	KMD-3310	MD 127	Others
Liver	0.5 h	1947.7 (100.0)	27.3 (1.4)	27.3 (1.4)	490.8 (25.2)	N.D. (N.D)	N.D. (N.D)	56.5 (2.9)	114.9 (5.9)	420.7 (21.6)	111.0 (5.7)	699.2 (35.9)
	4 h	1744.4 (100.0)	69.8 (4.0)	66.3 (3.8)	704.7 (40.4)	43.6 (2.5)	36.6 (2.1)	108.2 (6.2)	104.7 (6.0)	127.3 (7.3)	120.4 (6.9)	362.8 (20.8)
Kidneys	0.5 h	962.4 (100.0)	36.6 (3.8)	8.7 (0.9)	N.D. (N.D)	5.8 (0.6)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	666.9 (69.3)	N.D. (N.D)	244.4 (25.4)
	4 h	482.3 (100.1)	61.3 (12.7)	51.6 (10.7)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	247.9 (51.4)	N.D. (N.D)	122.0 (25.3)
Prostate	0.5 h	40.0 (100.1)	10.7 (26.7)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	21.4 (53.6)	N.D. (N.D)	7.9 (19.8)
	4 h	59.3 (100.1)	19.2 (32.3)	11.0 (18.5)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	18.2 (30.7)	N.D. (N.D)	11.0 (18.6)
Plasma	0.5 h	111.8 (100.0)	5.4 (4.8)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	83.5 (74.7)	N.D. (N.D)	22.9 (20.5)
	4 h	60.4 (99.9)	7.2 (11.9)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	N.D. (N.D)	24.1 (39.9)	N.D. (N.D)	29.1 (48.1)

N.D.: Not detected

Each radioactivity concentration represents the mean value of 3 samples. (Concerning the prostate, 3 samples were pooled and 1 pooled sample was measured). Concentrations of unchanged drug and metabolites were calculated by multiplying the radioactivity concentration in the tissue by respective percentages of unchanged drug and metabolites. Values in parentheses represent respective percentages of unchanged drug and metabolites: 3 samples were pooled and 1 pooled sample was analyzed by RI-HPLC.

% of dose

	Sampling time	Total excretion Rate of radioactivity	KMD-3213	KMD-3241	KMD-3250	KMD-3289	KMD-3293	KMD-3295	KMD-3299	KMD-3310	MD 127	Others
Urine	0-24 h	20.5 (100.1)	2.6 (12.8)	2.0 (9.7)	N.D. (N.D)	0.3 (1.3)	N.D. (N.D)	0.2 (1.0)	N.D. (N.D)	7.8 (37.9)	N.D. (N.D)	7.6 (37.3)
Feces	0-24 h	68.0 (100.0)	8.6 (12.7)	6.3 (9.2)	21.5 (31.6)	N.D. (N.D)	N.D. (N.D)	6.2 (9.1)	6.5 (9.5)	N.D. (N.D)	N.D. (N.D)	19.0 (27.9)
Bile	0-24 h	70.1 (99.9)	1.6 (2.3)	4.6 (6.6)	18.2 (25.9)	0.6 (0.8)	2.0 (2.8)	11.3 (16.1)	2.5 (3.6)	1.3 (1.9)	N.D. (N.D)	28.0 (39.9)
Cage washings	0-24 h	0.9	–	–	–	–	–	–	–	–	–	–

N.D.: Not detected

Each excretion rate of radioactivity represents the mean value of 3 samples. [–] indicates that no measurement was performed. Excretion rates of unchanged drug and metabolites were calculated by multiplying the excretion rates of radioactivity by respective percentages of unchanged drug and metabolites. Values in parentheses represent respective percentages of unchanged drug and metabolites: 3 samples were pooled and 1 pooled sample was analyzed by RI-HPLC.

Effects of repeated oral administration of KMD-3213 on hepatic drug-metabolizing enzymes in male rats (KMD- YK-04).

The effects of KMD-3213 after repeated oral administration on rats at a dose of 1, 10, or 30 mg/kg once daily for 7 days on drug-metabolizing enzymes in hepatic microsomes were investigated.

All of indexes tested in this study except rat body weight increased significantly in positive control group, i.e. phenobarbital group (80 mg/kg, intraperitoneally).

Cytochrome P450 contents significantly increased in 10 and 30 mg/kg KMD-3213 groups and increases were by 1.12- and 1.23-fold of those in negative control group, respectively.

Aniline 4-hydroxylase activity and UDPG transferase activity decreased significantly in 10 or 1 mg/kg groups, respectively, however, those decreases were 0.83 or 0.78 to those in negative control group, respectively, and not dependent on dose administered.

There were no effects on microsomal protein content, aminopyrine N-demethylase activity, 7-ethoxycoumarin 0-deethylase activity, and glutathione S-transferase activity.

These results indicated that the effects of KMD-3213 on drug-metabolizing enzymes in rat liver are slight.

Effects of treatment with KMD-3213 for 7 days on hepatic microsomal drug-metabolizing enzymes in male rats (using Dunnett's test)

Parameter	KMD-3213 (mg/kg/day)				PB (mg/kg/day)
	control	1	10	30	80
Initial body weight (g)	214± 11	210± 11	217± 7	214± 10	216± 13
Body weight (g)	219± 13	218± 17	230± 8	221± 12	229± 18
Liver weight (g)	8.36± 0.54	7.88± 0.82	9.06± 0.19	8.65± 0.86	12.39± 1.04###
Liver weight (% of body weight)	3.82± 0.20	3.63± 0.33	3.95± 0.16	3.91± 0.31	5.44± 0.48###
Microsomal protein content					
(mg/g liver)	17.0± 1.1	18.7± 4.2	16.7± 1.2	18.2± 2.8	24.5± 2.4###
(mg/liver)	142± 13	146± 24	151± 11	157± 25	302± 27###
Cytochrome content (nmol/mg protein)					
Cytochrome P450	1.12± 0.09	1.24± 0.13	1.25± 0.05*	1.37± 0.14***	3.08± 0.26###
Cytochrome b5	0.49± 0.16	0.55± 0.15	0.45± 0.08	0.38± 0.05	0.84± 0.39##
Enzyme activity (nmol/min/mg protein)					
Aminopyrine N-demethylase	5.51± 0.50	5.88± 0.35	5.49± 0.73	5.97± 0.76	10.41± 0.54###
Aniline 4-hydroxylase	0.90± 0.13	0.89± 0.14	0.75± 0.08*	0.77± 0.12	1.71± 0.16###
7-Ethoxycoumarin O-deethylase	1.89± 0.17	2.00± 0.25	2.00± 0.29	1.96± 0.15	2.46± 0.10###
UDPG transferase	37.5± 7.6	29.1± 7.2*	36.2± 5.6	31.1± 5.0	52.9± 4.9###
Glutathione S-transferase	172± 20	173± 22	168± 19	173± 24	280± 19###

Data are expressed as the mean values±S.D. of eight animals.
PB: Phenobarbital sodium
Significantly different from the value of control by Dunnett's method : * ; $p<0.05$, ** ; $p<0.01$, *** ; $p<0.001$
Significantly different from the value of control by Student's t-test : # ; $p<0.05$, ## ; $p<0.01$, ### ; $p<0.001$

Effects of treatment with KMD-3213 for 7 days on hepatic microsomal drug-metabolizing enzymes in male rats (relative to control)

Parameter	KMD-3213 (mg/kg/day) 1	10	30	PB (mg/kg/day) 80
Initial body weight	0.98	1.01	1.00	1.01
Body weight	0.99	1.05	1.01	1.04
Liver weight (g)	0.94	1.08	1.03	1.48
Liver weight (% of body weight)	0.95	1.04	1.02	1.42
Microsomal protein content				
(mg/g liver)	1.10	0.98	1.07	1.44
(mg/liver)	1.02	1.06	1.10	2.12
Cytochrome content				
Cytochrome P450	1.11	1.12	1.23	2.76
Cytochrome b5	1.11	0.91	0.78	1.72
Enzyme activity				
Aminopyrine N-demethylase	1.07	1.00	1.08	1.89
Aniline 4-hydroxylase	0.99	0.83	0.86	1.90
7-Ethoxycoumarin O-deethylase	1.06	1.06	1.04	1.30
UDPG transferase	0.78	0.97	0.83	1.41
Glutathione S-transferase	1.00	0.98	1.01	1.62

Mean values of eight animals were used to calculate values in Table 3.
PB: Phenobarbital sodium

2.6.4.6 Excretion

Pharmacokinetics: Excretion

Rats: Single Dose Test Article: Silodosin

In fasting rats given a single oral administration of ^{14}C-KMD-3213 at 1 mg/kg, urine, feces, and expired air accounted for 15.3%, 81.7%, and 0.5%, respectively, of the total administered radioactivity 168 hours after administration.

Species/strain:	Rats/SD	Dose (mg/kg):	1
Sex (M/F/N=	M/3	Substance to be assayed (target substance):	TRA
Vehicle/dosing form:	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)	Assay method:	LSC
Administration method:	Forced oral dose		
Study No.:	AE-3514-G		

Feeding	Fasting				Non-fasting			
Route of excretion	Cumulative excretion rate (%)				Cumulative excretion rate (%)			
	Urine	Feces	Expiration	Total	Urine	Feces	Expiration	Total
Time								
0 – 4	1.1 ± 1.0	–	0.1 ± 0.1	–	0.9 ± 0.7	–	0.0 ± 0.0	–
0 – 8	8.3 ± 2.7	–	0.2 ± 0.1	–	3.2 ± 1.5	–	0.1 ± 0.0	–
0 – 24	13.5 ± 5.6	72.3 ± 5.2	0.4 ± 0.1	86.2 ± 2.4	5.8 ± 0.8	76.7 ± 4.6	0.2 ± 0.0	82.7 ± 4.4
0 – 48	14.6 ± 5.8	79.7 ± 4.9	0.4 ± 0.1	94.7 ± 2.7	6.5 ± 0.8	90.2 ± 2.2	0.3 ± 0.1	97.0 ± 1.4
0 – 72	15.2 ± 6.3	81.4 ± 3.7	0.5 ± 0.1	97.1 ± 3.9	6.6 ± 0.8	90.7 ± 2.2	0.3 ± 0.1	97.6 ± 1.4
0 – 96	15.3 ± 6.4	81.5 ± 3.7	0.5 ± 0.1	97.3 ± 4.1	6.7 ± 0.8	90.7 ± 2.2	0.3 ± 0.1	97.7 ± 1.3
0 – 120	15.3 ± 6.4	81.6 ± 3.6	0.5 ± 0.1	97.4 ± 4.2	6.7 ± 0.8	90.8 ± 2.2	0.3 ± 0.1	97.7 ± 1.4
0 – 144	15.3 ± 6.4	81.6 ± 3.6	0.5 ± 0.1	97.5 ± 4.4	6.7 ± 0.8	90.8 ± 2.2	0.3 ± 0.1	97.7 ± 1.4
0 – 168	15.3 ± 6.4	81.7 ± 3.6	0.5 ± 0.1	97.6 ± 4.4	6.7 ± 0.8	90.8 ± 2.2	0.3 ± 0.1	97.8 ± 1.3
Cadaver				0.6 ± 0.2				0.2 ± 0.1

LSC: Liquid scintillation counter, TRA: Total radioactivity,
Data represents mean ± S.D., –: Not measured

Rats: Repeated Dose Test Article: Silodosin
In non-fasting rats receiving repeat oral administration of ^{14}C-KMD-3213 at 1 mg/kg once daily for 21 days, urinary and fecal excretion fractions were 9.9% and 87.4%, respectively, of the administered radioactivity and 97.3% in total. In addition, the urinary excretion fraction was constant 12 days after administration.

Species/strain:	Rats/SD	Sex (M/F)/N=	M/3
Feeding:	Non-fasting		
Vehicle/dosing form:	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)		
Administration method:	Forced oral		
Dose (mg/kg/day):	1 (repeated doses for 21 days)		
Substance to be assayed (target substance):	TRA	Assay method:	LSC
Study No.:	KMD-PKM502		

Route of excretion	Cumulative excretion rate (%)		
	Urine	Feces	Total
Time(Day)			
1	7.7 ± 2.5	75.8 ± 14.9	83.5 ± 15.9
2	7.3 ± 2.5	77.2 ± 3.8	84.5 ± 4.5
3	7.6 ± 3.4	80.4 ± 1.8	88.0 ± 3.3
4	8.1 ± 2.7	81.6 ± 3.3	89.7 ± 0.7
5	8.3 ± 2.0	83.0 ± 1.8	91.3 ± 1.2
6	8.8 ± 2.5	83.5 ± 0.7	92.2 ± 1.8
7	8.7 ± 1.9	84.0 ± 2.6	92.8 ± 4.4
8	8.8 ± 1.5	84.1 ± 1.4	92.9 ± 2.9
9	9.0 ± 1.3	86.4 ± 3.2	95.4 ± 4.3
10	9.0 ± 1.3	86.1 ± 2.1	95.1 ± 3.2
11	9.1 ± 1.0	87.0 ± 3.0	96.1 ± 3.3
12	9.7 ± 0.9	87.9 ± 4.4	97.6 ± 5.2
13	9.8 ± 0.9	88.5 ± 3.4	98.2 ± 4.2
Time(Day)			
14	9.8 ± 0.9	88.2 ± 2.3	98.0 ± 3.1
15	9.9 ± 0.8	88.2 ± 3.2	98.1 ± 3.8
16	9.9 ± 0.9	88.4 ± 3.0	98.3 ± 3.5
17	9.9 ± 0.8	88.1 ± 3.6	98.0 ± 4.2
18	9.9 ± 0.8	87.4 ± 4.2	97.3 ± 4.8
19	9.8 ± 0.7	86.9 ± 3.8	96.7 ± 4.3
20	9.8 ± 0.7	87.2 ± 3.7	97.0 ± 4.3
21	9.7 ± 0.4	86.9 ± 3.3	96.6 ± 3.6
22 (1 day after the end of treatment)	9.8 ± 0.4	87.3 ± 3.5	97.1 ± 3.9
23 (2 day after the end of treatment)	9.8 ± 0.4	87.4 ± 3.5	97.2 ± 3.9
24 (3 day after the end of treatment)	9.8 ± 0.4	87.4 ± 3.5	97.2 ± 3.9
25 (4 day after the end of treatment)	9.9 ± 0.4	87.4 ± 3.5	97.3 ± 3.9
26 (5 day after the end of treatment)	9.9 ± 0.4	87.4 ± 3.5	97.3 ± 3.9
27 (6 day after the end of treatment)	9.9 ± 0.4	87.4 ± 3.5	97.3 ± 3.9
28 (7 day after the end of treatment)	9.9 ± 0.4	87.4 ± 3.5	97.3 ± 3.9

Data represents mean ± S.D.

Dogs: Single Dose Test Article: Silodosin
In fasting dogs administered a single oral administration of ^{14}C-KMD-3213 at 0.5 mg/kg, radioactivity excreted in urine and feces, and recovered from the cage wash, was 24.62%, 61.18%, and 3.73%, respectively, of the administered radioactivity, and 89.53% in total.

Species/strain:	Dogs/beagle	Dose (mg/kg):	0.5
Sex (M/F)/N=	M/3	Substance to be assayed (target substance):	TRA
Feeding:	Fasting	Assay method:	LSC
Vehicle/dosing form:	Capsule (dissolved in dilute hydrochloric acid)		
Administration method:	Oral administration		
Study No.:	KSI 137/013760		

Route of excretion	Cumulative excretion rate (%)		
	Urine	Feces	Washing Solution
Time			
0 - 12	19.44 ± 9.85	–	–
0 - 24	22.51 ± 9.31	43.06 ± 11.13	1.74 ± 0.81
0 - 48	23.46 ± 9.43	58.17 ± 10.33	2.87 ± 0.31
0 - 72	24.03 ± 9.73	59.15 ± 10.68	3.08 ± 0.28
0 - 96	24.27 ± 9.80	59.61 ± 10.75	3.22 ± 0.28
0 - 120	24.40 ± 9.78	60.03 ± 10.78	3.33 ± 0.24
0 - 144	24.50 ± 9.79	60.31 ± 10.88	3.43 ± 0.26
0 - 168	24.62 ± 9.82	61.18 ± 11.06	3.73 ± 0.33
Total			89.53 ± 1.31

LSC: Liquid scintillation counter.
TRA: Total radioactivity.
Data represents mean ± S.D.
-: Not measured.

Humans: Single Dose Test Article: Silodosin

This mass balance study in which healthy male volunteers received a single oral administration of 8 mg of ^{14}C-KMD-3213, revealed urinary and fecal excretion fractions that accounted for 33.5% and 54.9%, respectively, of the administered radioactivity, and 88.4% in total.

Species:	Humans	Dose:	8 mg
Sex (M/F)/N=	M/6	Substance to be assayed (target substance):	TRA
Feeding:	Fasting	Assay method:	LSC
Vehicle/dosing form:	Solution in dilute hydrochloric acid		
Administration method:	p.o.		
Study Number:	KMD3213-US012-99		

Route of excretion	Cumulative excretion rate (%)	
	Urine	Feces
Time		
0 - 6	16.9 ± 3.91	–
0 - 12	24.4 ± 4.23	–
0 - 24	28.9 ± 4.47	8.30 ± 8.58
0 - 48	31.8 ± 4.62	25.3 ± 21.0
0 - 72	32.7 ± 4.73	41.0 ± 16.9
0 - 96	33.1 ± 4.81	44.8 ± 15.9
0 - 120	33.3 ± 4.85	51.6 ± 12.4
0 - 144	33.4 ± 4.88	53.8 ± 11.7
0 - 168	33.4 ± 4.89	54.6 ± 11.6
0 - 192	33.4 ± 4.89	54.7 ± 11.6
0 - 216	33.5 ± 4.91	54.8 ± 11.6
0 - 240	33.5 ± 4.91	54.9 ± 11.6
Fecal wipe		0.07 ± 0.03
Total		88.4 ± 10.7

LSC: Liquid scintillation counter, TRA: Total radioactivity.
Data represents mean ± S.D.
-: Not measured.

Pharmacokinetics: Excretion into Bile Test Article: Silodosin

Species/strain: Rats/SD
Sex (M/F)/N= M/3
Feeding: Fasting
Substance to be assayed (target substance): TRA
Assay method: LSC
Study Number: AE-3514-G

Vehicle/dosing form:	0.5% Aqueous solution of methyl cellulose (dissolved in dilute hydrochloric acid)			Bile [a]		
Administration method:	Forced oral			Intraduodenal		
Dose (mg/kg):	1			0.0164 mg Eq/body		
Route of excretion	Cumulative excretion rate (%)			Cumulative excretion rate (%)		
	Bile	Urine	Feces	Bile	Urine	Feces
Time (hours)						
0 – 1	6.4 ± 7.1	–	–	0.5 ± 0.2	–	–
0 – 2	15.2 ± 11.4	–	–	3.2 ± 1.4	–	–
0 – 4	32.7 ± 7.3	9.3 ± 4.0	–	7.5 ± 2.1	5.7 ± 1.9	–
0 – 8	39.0 ± 7.5	14.3 ± 3.4	–	12.5 ± 2.3	9.2 ± 1.3	–
0 - 24	42.2 ± 6.6	16.9 ± 2.3	34.1 ± 9.7	15.2 ± 2.6	11.4 ± 1.2	60.5 ± 4.7
0 - 48	42.9 ± 6.6	17.4 ± 2.4	35.4 ± 9.3	15.9 ± 2.6	11.8 ± 1.3	69.1 ± 3.7
Gastrointestinal content (48)			2.3 ± 1.1			2.0 ± 1.1
Cadaver (48)			1.5 ± 0.2			0.7 ± 1.3

a) Bile pooled in 8 hours after oral dose of ^{14}C-KMD-3213 (1 mg/kg) in rats.
LSC: Liquid scintillation counter, TRA: Total radioactivity.
Data represents mean ± S.D.
–: Not measured.

2.6.4.7 Pharmacokinetic drug interactions: NA

2.6.4.8 Other Pharmacokinetic Studies: NA

2.6.4.9 Discussion and Conclusions

Repeat dose (14-day) oral absorption studies were conducted in mice, rats and dogs to evaluate levels of silodosin and its metabolites in the plasma. Not all metabolites were detected in the plasma of all species tested. In mice and rats, AUC and C_{max} values increased in a dose-dependent manner for both silodosin and its metabolites. In dogs, AUC and C_{max} values were higher in males compared to females for both silodosin and its metabolites. A summary of pharmacokinetic findings is found below.

Toxicokinetic studies were also conducted in conjunction with pivotal toxicology studies and indicated that silodosin and its metabolites were adequately included in those studies, except for the glucuronidated metabolite which circulates at approximately four times the plasma levels of silodosin in humans. Genetic toxicology studies (*in vitro*) and intravenous studies were conducted to qualify the major human glucuronidated metabolite KMD-3213G, which is not produced by mice, rats, or dogs (see Toxicology section).

In fasting rats receiving a single oral administration of 1 mg/kg ^{14}C-KMD-3213, radioactivity was rapidly distributed into the organs and tissues. High concentrations of radioactivity were determined 30 minutes after administration in the liver, kidneys, and

bladder in addition to the intestinal tract. High concentrations of radioactivity were determined in the liver and kidneys, and comparatively high concentrations were in the pituitary, the pancreas and the bladder 4 hours after administration. At 24 hours post-dose, the highest concentration of radioactivity was determined in the liver and the second highest concentration was in the pituitary. The radioactive concentrations decreased with time in most of the organs and tissues. Higher radioactivity was determined in the liver and the kidneys than in the other organs and tissues 168 hours after administration. Radioactivity disappeared more rapidly from plasma compared to other organs and tissues, and at 168 hours after administration, radioactivity was below the detection level in plasma. In the prostate, concentrations of radioactivity were as high as the concentrations in plasma, with radioactivity being detected even 168 hours after administration. In addition, the elimination half-lives of radioactivity in the testis, brown fat, white fat, skin, and heart were longer than those in other organs and tissues. Throughout all the time points, radioactive concentrations in the cerebrum and the cerebellum were lower than those in plasma. The distribution of silodosin to the central nervous system was low. In non-fasting rats given repeat oral administrations of ^{14}C-KMD-3213 at 1 mg/kg once daily for 21 days, high concentrations of radioactivity were distributed in the liver, followed by the kidneys, pituitary, and skin, in a manner similar to distribution following a single dose.

In vitro metabolism studies using rat, dog, monkey, and human hepatocytes showed that a glucuronide conjugate of silodosin (KMD-3213G) and a metabolite in which the alcoholic hydroxy group was oxidized (KMD-3293) were mainly synthesized in hepatocytes and are the major metabolites in humans. KMD-3293 was also synthesized in rat, dog, and monkey hepatocytes, but the major human metabolite KMD-3213G was not seen in rat or dog hepatocytes and was minimal in monkey hepatocytes. In repeat (14-day) oral administration of silodosin to mice, rats, and dogs, the major metabolites for each test species were measured. In mice and rats, AUC and C_{max} values increased in a dose-dependent manner for both silodosin and its metabolites. In dogs, AUC and C_{max} values were higher in males compared to females for both silodosin and its metabolites. In healthy male human volunteers given a single oral administration of 8 mg of ^{14}C-KMD-3213, KMD-3213G and KMD-3293 were mainly detected in plasma, along with silodosin. KMD-3213G was not detected in plasma after a single oral administration of ^{14}C-KMD-3213 at 1 mg/kg and 0.5 mg/kg in rats and dogs, respectively. KMD-3293 was low in concentration in rat and dog plasma after the same single oral administration conditions as above. However, single oral administration toxicity studies in which silodosin was administered to mice at 100 and 500 mg/kg, to rats at 300 and 600 mg/kg, and to dogs at 100 and 200 mg/kg showed that KMD-3293 was detected in rat and dog plasma at concentrations in excess of the observed concentrations of KMD-3293 in humans that received a single oral administration of 8 mg of silodosin. In rat and dog plasma, KMD-3310, which has a lower affinity for α_1-ARs, was the major metabolite. In the prostate of rats 30 minutes after administration, KMD-3310 was the major metabolite, followed by silodosin. At 4 hours after administration the percentage of silodosin in the rat prostate was greater than that of KMD-3310. Both silodosin and KMD-3310 were present up to 4 hours post-dose indicating that the product was present at the therapeutic site. KMD-3310 was detected in rat and dog urine. KMD-3310 and KMD-3293 were

mainly detected in human urine. Silodosin was detected in the urine of all species but accounted for not more than 4% of the administered dose. KMD-3250 was mainly detected in rat feces, and KMD-3293 and KMD-3295 were mainly noted in dog feces. KMD-3293 was mainly detected in human feces. Human fecal excretion of silodosin accounted for about 30% of the administered dose. KMD-3213G, the major metabolite of silodosin in human plasma, was not detected in human feces. It was inferred that KMD-3213G was de-conjugated by enteroenzymes and excreted into feces as silodosin. KMD-3250 was mainly detected in the rat liver and bile, while KMD-3310 was detected in rat kidneys. On the basis of these results, KMD-3310 was considered the major metabolite in rat and dog plasma, and KMD-3213G and KMD-3293 were the major metabolites in human plasma.

^{14}C-KMD-3213 was primarily excreted in feces in rats, dogs, and humans. In fasting, bile duct cannulated rats (single oral administration of ^{14}C-KMD-3213 at 1 mg/kg), biliary excretion accounted for 42.9% of the administered radioactivity, with urinary and fecal excretion accounting for 17.4% and 35.4%, respectively, up to 48 hours after administration. Therefore, silodosin was considered to be primarily excreted via bile into feces.

2.6.4.10 Tables and figures to include comparative TK summary

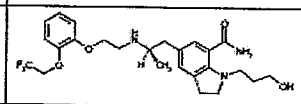
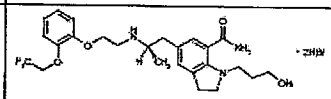
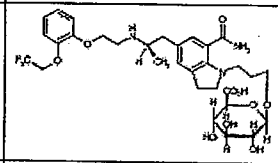
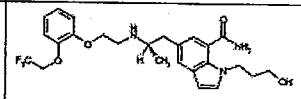
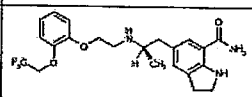
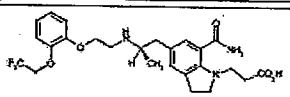
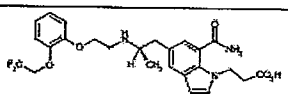
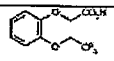
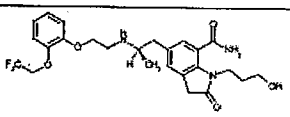
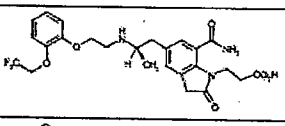
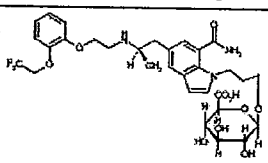
Comparison of Maximum Plasma Drug Concentrations (Cmax) and Systemic Exposures (AUC_{0-24}) of Silodosin and Metabolites at Steady State between Animals and Humans (see discussion above).

Species	Sex	Dose (mg/kg)	Silodosin		KMD-3289		KMD-3241		KMD-3293		KMD-3295		KMD-3310	
			C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}	C_{max}	AUC_{0-24}
Mouse	Male	20	17	226	6	80	2	23	2	7	8	70	NA	NA
		60	137	1,459	43	479	30	270	20	202	39	464	NA	NA
		100	303	3,284	99	1,225	54	617	67	431	95	723	NA	NA
	Female	60	160	1,957	31	462	26	331	83	443	105	921	NA	NA
		150	772	10,683	229	3,421	168	2,208	761	2,924	735	4,020	NA	NA
		400	1,727	26,827	642	9,518	305	4,763	912	6,126	565	4,703	NA	NA
Rat	Male	15	13	203	0	2	3	34	3	14	NA	NA	40	426
		50	81	942	28	254	40	407	15	158	NA	NA	177	1,912
		150	228	2,947	115	1,417	135	1,607	43	523	NA	NA	383	5,288
	Female	15	13	163	0	3	3	38	0	0	NA	NA	27	279
		80	106	1,194	24	277	37	463	11	105	NA	NA	131	1,600
		250	352	3,078	76	817	90	993	41	315	NA	NA	262	3,275
Dog	Male	80	5,303	36,977	1,109	13,869	1,387	13,646	178	911	67	384	6,947	74,911
	Female	80	3,477	22,065	674	7,535	1,023	7,958	153	711	37	177	5,077	56,760
Human	Male	8 mg	61.6	373.4	NA	NA	NA	NA	34.3	373.0	3.4	16.8	1.6	2.8

1) ng/mL.
2) ng·hr/mL.
NA: Not applicable.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

List of compounds in pharmacokinetic studies:

Abbreviation • generic name	Chemical name	Structural formula	Origin
KMD-3213 silodosin	1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl}amino)propyl]-2,3-dihydro-1*H*-indole-7-carboxamide		Drug substance
KMD-3213•2HBr salt	1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl}amino)propyl]-2,3-dihydro-1*H*-indole-7-carboxamide dihydrobromide		Dihydro-bromid acid salt of the drug substance
MD127 (glucuronide conjugate of silodosin or KMD-3213G)	3-{7-Carbamoyl-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoro-ethoxy)phenoxy]ethyl}-amino)propyl]-2,3-dihydro-1*H*-indol-1-yl}propyl β-D-glucopyranosiduronic acid		Metabolite
KMD-3241	1-(3-Hydroxypropyl)-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl}amino)propyl]-1*H*-indole-7-carboxamide		Metabolite
KMD-3289	5-[(2*R*)-2-({2-[2-(2,2,2-Trifluoroethoxy)phenoxy]-ethyl}amino)propyl]-2,3- dihydro-1*H*-indole-7-carboxamide		Metabolite
KMD-3293	7-Carbamoyl-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl}amino) propyl]-2,3-dihydro-1*H*- indole-1-propanoic acid		Metabolite
KMD-3295	7-Carbamoyl-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl}amino) propyl]-1*H*-indole-1- propanoic acid		Metabolite
KMD-3310	2-(2,2,2-Trifluoroethoxy)-phenoxyacetic acid		Metabolite
KMD-3250	1-(3-Hydroxypropyl)-2-oxo-5-[(2*R*)-2-({2-[2-(2,2,2trifluoroethoxy)-phenoxy]ethyl}amino)-propyl]-2,3-dihydro-1*H*-indole-7-carboxamide		Metabolite
KMD-3299	7-Carbamoyl-2-oxo-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoroethoxy)phenoxy]-ethyl}amino)propyl]-2,3-dihydro-1*H*-indole-1-propanoic acid		Metabolite
KMD-3241 glucuronide conjugate (KMD-3241G)	3-{7-Carbamoyl-5-[(2*R*)-2-({2-[2-(2,2,2-trifluoro-ethoxy)phenoxy]ethyl}-amino)propyl]-1*H*-indol-1-yl}propyl β-D-glucopyranosiduronic acid		Metabolite

Pharmacokinetic Overview

Type of Study	GLP	Test System	Method of Administration	Testing Facility	Study Number
Absorption					
Absorption	No	Rats	Forced oral, Intravenous	Kissei	PK10152
Absorption	No	Rats	Forced oral	Kissei	PK10066
Absorption	No	Rats	Intravenous	Kissei	PK10053
Absorption, metabolism and excretion	Yes	Dogs	Forced oral	[redacted]	KSI 137/013760
Absorption	No	Dogs	Forced oral, Intravenous	Kissei	KMD-YA-8
Absorption, metabolism and excretion	No*	Humans	p.o. (solution in dilute hydrochloric acid)	[redacted]	KMD3213-US012-99
Absorption	No*	Humans	p.o., Intravenous	[redacted]	KMD-308
Absorption	No*	Humans	p.o.	[redacted]	98363
Absorption	No*	Humans	p.o.	[redacted]	KMD-105
Absorption	No*	Humans	p.o.	[redacted]	SI06004
Absorption	Yes	Mouse	p.o.	[redacted]	RTI-989 / 07-8989
Absorption	Yes	Rat	p.o.	[redacted]	RTI-988 / 07-8988
Absorption	Yes	Dog	p.o.	[redacted]	RTI-987 / 07-8987
Absorption site	No	Rats	Injection into the gastrointestinal tract	Kissei	PK10149

[redacted], Kissei: Kissei Pharmaceutical Co., Ltd.

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* Followed GCP guidelines.

Type of Study	GLP	Test System	Method of Administration	Testing Facility	Study Number
Distribution					
Tissue distributions after single dose	No	Rats	Forced oral	[redacted]	AE-3513-G
Tissue distributions after single dose (ARG)	Yes	Rats	Forced oral	[redacted]	KSI 58/961783
Tissue distributions after repeated dose	No	Rats	Forced oral	Kissei	KMD-PKM501
Plasma protein binding	No	Rats, Dogs, Humans (plasma protein)	*In vitro*	Kissei	PK10153
Plasma protein binding (Glucuronide conjugate of KMD-3213)		Rats, Dogs, Humans (plasma protein)	*In vitro*	Kissei	DMPK2003-0053
Plasma protein binding (KMD-3293)		Humans (plasma protein)	*In vitro*	Kissei	DMPK2004-0033
Concentration in blood cells	No	Rat blood	*In vitro*	Kissei	PK10151
Concentration in blood cells	Yes	Dog blood	*In vitro*	[redacted]	KSI 137/013760
Concentration in blood cells		Human blood	*In vitro*	Kissei	PK10091

[redacted], Kissei: Kissei Pharmaceutical Co., Ltd.

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Type of Study	GLP	Test System	Method of Administration	Testing Facility	Study Number
Metabolism					
Metabolism in hepatocytes	No	Rats, dogs, humans	*In vitro*	Kissei	PK10104
Metabolism in hepatocytes	No	Monkeys	*In vitro*	Kissei	PK-03-519
Metabolites in plasma, urine, feces, bile and organs		Rats	Forced oral	Kissei	PK10055
Metabolites in plasma, urine and feces	Yes	Dogs	Forced oral	[redacted]	KSI 137/013760
Metabolites in plasma, urine and feces	No*	Humans	p.o.	[redacted]	KMD3213-US012-99
Effects on hepatic drug metabolizing enzymes (enzyme induction)	No	Rats	Forced oral	Kissei	KMD-YK-04
Excretion					
Excretion after single dose		Rats	Forced oral	[redacted]	AE-3534-G
Excretion after repeated dose		Rats	Forced oral	Kissei	KMD-PKM502
Absorption, metabolism and excretion	Yes	Dogs	Forced oral	[redacted]	KSI 137/013760
Absorption, metabolism and excretion	No*	Humans	p.o.	[redacted]	KMD3213-US012-99
Bile excretion		Rats	Forced oral	[redacted]	AE-3534-G
Enterohepatic circulation		Rats	Injection into the duodenal	[redacted]	AE-3534-G

[redacted] Kissei: Kissei Pharmaceutical Co., Ltd. [redacted]

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* Followed GCP guidelines.

Toxicokinetic Overview:

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, 250	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.

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Overview of toxicokinetic data:

AUC (ng • hr/mL) of silodosin at steady stage

Daily Dose (mg/kg)	Mice [a)]		Rats [b)]		Dogs [c)]		Rabbits [b)]	Humans [h)]
	Male	Female	Male	Female	Male	Female	Female	Male
0.11								373±165
5					1082±1091	582±250		
10					3350±962	3063±1156		
15			422±81 [f)]	261±62 [f)]				
20	229±42		701	532	7301±591	4628±3181	209 ± 69	
25					31139±15131	24188±11156		
30			1998 d)	1597 [d)]				
50			2011±193 [g)]		30096±11116	25627±6153		
60	1897±340	2543±435	2568	1858			1131 ± 210	
80				2235±181 [g)]	19147±9096	44097±8534		
100					158780±45600 [e)], 39327 [f)]	122309±29482 [e)], 38975±9150 [f)]		
125			7570 [d)]	3814 [d)]				
150		16298±2624	9509±707 [g)]					
200	15044 [d)] 22810±2398 [g)]	15298	11151	4106			9056 ± 2268	
250				8282±557 [g)]				
400	29288	43494 [g)], 64058±6149 [g)]			157039	159359		
500			27592 [d)]	12254 [d)]				
600			24241	7519				
800	41139	80077						

a) Mixed with food, b) Forced oral administration, c) Capsules, d) Mixed with food, 13-week dose-finding study, e) 1-month dose study, f) 13-week dose study, g) Mixed with food, 104-week carcinogenicity study, h) Phase I repeated dose study SI06004.

Intravenous injection studies

Daily Dose (mg/kg)	Rats				Dogs				Humans [e)]
	Male		Female		Male		Female		Male
$T_{1/2}$ (h)									
0.04									3.6 ± 1.7
25					2.11 ± 0.82 [a)]				
50					2.81 ± 0.49 [a)]				

a) Single dose study, e) Clinical pharmacological study KMD-308.

Listing of the Investigation Product Lots used in the Toxicology Studies

Batch No.	Purity (%)	Specified Impurities (%) [a)]			Study No.	Type of Study
		[redacted]	Others (individual)	Total		
Specification in application:	[redacted]	[redacted]	[redacted]	[redacted]		
FP151	100.1	[redacted]	[redacted] Others max [redacted]	[redacted]	00229	Single oral dose study in rats (1)
					00233	Single oral dose study in dogs (1)
GD231	99.6	N.D.	Max : [redacted]	N.C.	10017	Single oral dose study in rats (2)
					10025	Single oral dose study in dogs (2)
					10077	3-month oral dose study in rats
					10008	1-month oral dose study and 1-month recovery study in dogs
					KSI 70/970908	13-week oral dose study in dogs
					10036	Reverse mutation test in bacteria
					2626 (005-013)	Chromosomal aberration test with mammalian cells in culture (1)
					10067	Micronucleus test in mice
					10006	Study on fertility and early embryo development until implantation in rats
					10072	Study on fertility and early embryo development until implantation in rats – study in female rats
					10037	Antigenicity study in mice
					10069	Antigenicity study in guinea pigs

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Batch No.	Purity (%)	Specified Impurities (%) [a]			Study No.	Type of Study
			Others (individual)	Total		
Specification in application:						
GL091	99.7		Max		10026	1-month oral dose study and 1-month recovery study in rats
					10081	26-week oral dose study in rats (1)
					KSI 70/970908	13-week oral dose study in dogs
GT081	99.6		Max		10059	Study on fertility and early embryo development till implantation in rats – study inmale rats (1)
					10058	Embryo - fetal development study in rats (1)
HZ271	100.0		Max		10050	Embryo - fetal development study in rabbits
HH231	99.7		Max		10081	26-week oral dose study in rats (1)
GX201	99.8		Max		KSI 71/974423	52-week oral dose study in dogs
JH312	100.5	N.D.	N.D.	N.D.	10092	Single intravenous injection study in rats
					10093	Single intravenous injection study in dogs
					10081	26-week oral dose study in rats (1)
					10111	26-week oral dose study in rats (2)
					KSI 80/973223	Mouse lymphoma assay
					7L425	Chromosomal aberration test with mammalian cells in culture (2)
					KSI 083/974372	Unscheduled DNA synthesis (UDS) test with rat hepatocytes
					10112	Study on fertility and early embryo development till implantation in rats – study in male rats (2)
					10101	Study of pre- and post-natal development and function of dams in rats

Batch No.	Purity (%)	Specified Impurities (%) [a]			Study No.	Type of Study
			Others (individual)	Total		
Specification in application:						
JJ141	100.2	N.D.	N.D.	N.D.	KSI 089/982293	14-day palatability study in mice
					KSI 088/982292	14-day palatability study in rats
JL201	99.9	N.D.	N.D.	N.D.	KSI 086/982491	13-week dose-finding study in mice
					KSI 084/982477	13-week dose-finding study in rats
JN301	100.0				KSI 100/012988	104-week carcinogenicity study in female mice
					KSI 114/012990	104-week carcinogenicity study in male mice
					KSI 102/012989	104-week carcinogenicity study in rats
					10140	Embryo - fetal development study in rats (2)
T02090505	100.1				KMD 3213-IT-PH 0233	28-day toxicity study in male rats with a 30-day recovery period
					KMD 3213-IT-PH 0236	*In vitro* phototoxicity

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Batch No.	Purity (%)	Specified Impurities (%) [a]			Study No.	Type of Study
		[redacted]	Others (individual)	Total		
Specification in application:	[redacted]					
JR261	100.2	N.D.	N.D.	N.D.	KSI 086/982491	13-week dose-finding study in mice
					KSI 100/012988	104-week carcinogenicity study in female mice
					KSI 114/012990	104-week carcinogenicity study in male mice
					KSI 084/982477	13-week dose-finding study in rats
					KSI 102/012989	104-week carcinogenicity study in rats
					10116	Oral dose toxicokinetic study in female rabbits
JZ011	100.5	[redacted]	[redacted]	N.C.	10242	2-week intravenous injection study in rats
					10236	2-week intravenous injection study in dogs
					KSI 114/012990	104-week carcinogenicity study in male mice
					KSI 102/012989	104-week carcinogenicity study in rats
					10281	*In vitro* study of inhibitory effect on trypsin
					10282	*In vitro* study of inhibitory effect on papain
					10235	Study on metabolites after single oral dose in mice
					10216	Study on metabolites after single oral dose in rats
					10221	Study on metabolites after single oral dose in dogs
					10219	Study on blood hormone levels after oral dose in rats
					10220	Study on blood hormone levels after oral dose in mice
					10283	Study on hypertrophy of the thyroid by repeated oral dose in rats
					KSI 115/994798	4-week oral dose study to investigate accumulation of metabolites in the liver and kidney in dogs

Batch No.	Purity (%)	Specified Impurities (%) [a]			Study No.	Type of Study
		[redacted]	Others (individual)	Total		
Specification in application:	[redacted]					
KMD060781	99.9	[redacted]	[redacted]	[redacted]	RTI-897	Study of metabolites after 14-day dosing to dogs
					RTI-898	Study of metabolites after 14-day dosing to rats
					RTI-899	Study of metabolites after 14-day dosing to mice
Batch No.	Purity (%)				Study No.	Type of Study
Specification:	[redacted]					
NV231	[redacted]				2002IF006	Hemolysis study in human blood
					2002IF005	Intramuscular injection study in rabbits

a) Content (%).
N.D. = Not detected.
N.C. = Not calculated.

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2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

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) in dogs. 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 positive for neutral lipids. At 80 mg/kg/day (about 51 – 118 times), pharmacological signs (prominent third eyelid, eyes reddened/glazed, unsteady gait, stiff hind leg gait, high stepping gait, trembling, subdued behavior/lying down, liquid feces) were observed for the duration of the study, 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 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 (about 5 times the expected clinical exposure). At 50 mg/kg/day (about 64 times), 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 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 two-week intravenous toxicity study of the major human glucuronidated metabolite, MD127K (KMD-3213G), the metabolite was found to be similar both in pharmacology and toxicology to KMD-3213. Pharmacology studies showed this metabolite to be slightly less active than the parent drug, and distribution studies showed it to be distributed to tissue, including the prostate.

Genetic toxicology:

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 silodosin tested, and it was concluded that it was not genotoxic under the conditions tested in a mammalian cell mutation assay

.

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.

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.

Carcinogenicity:

In a carcinogenicity study by dietary administration to CD-1 mice for 104 weeks mammary gland adenoacanthomas, mammary gland adenocarcinomas, and mammary gland adenomas or carcinomas were statistically significant and were considered drug related. 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 treatment related neoplasms. Mice do not produce the major human glucuronidated metabolite.

In a carcinogenicity study by dietary administration to CD rats for 104 weeks, 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. Rats do not produce the major human glucuronidated metabolite.

Reproductive toxicology:

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 group. Histopathological examination of infertile males revealed changes in the testes and epididymides in the 200 and 600 mg/kg groups. Reduction in fertility in treated groups did not reach statistical significance in this study, as it did in two other studies. However, the fertility index was somewhat lower in the 600 mg/kg group, and implantation index observed at cesarean section was significantly lower.

In an additional male rat fertility study, a decrease in fertility and implantation index was observed in the 20 mg/kg/day group. Effects on fertility and implantation indices

recovered after a 2 week recovery period. No effects of KMD-3213 on reproductive function were observed in the 2 and 6 mg/kg/day groups.

In a female rat fertility study, no effect on fertility parameters was observed at 20 mg/kg/day. However, a minor, but significant effect on estrus cycling was observed at 20 mg/kg/day with a no effect level at 6 mg/kg/day.

Special toxicology:

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.

2.6.6.2 Single-dose toxicity

A single dose oral administration study of silodosin in rats showed that the approximate lethal dose was 800 mg/kg for males and females and the LD_{50} value was estimated at 878 mg/kg for both males and females. The approximate lethal doses for male and female rats by intravenous administration were 75 mg/kg and 90 mg/kg, respectively. A single dose study in dogs showed that the approximate lethal dose was 1500 mg/kg for orally administered silodosin and 50 mg/kg or more via intravenous injection. KMD-3213G (MD127K) was lethal at 50 mg/kg/day by intravenous administration in both male and female rats.

2.6.6.3 Repeat-dose toxicity

Study title: Twenty-six-week oral toxicity study of KMD-3213 in rats

Key study findings:

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.

Study no.: 10081
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: 13 November, 1996
GLP compliance: Japanese Ministry of Health and Welfare
QA report: yes (x) no ( )
Drug, lot #, and % purity: Lot nos. GL091 (99.0 % pure), HZ271 (100.0 % pure), JH312 (100.5 % pure)

Methods

Doses: 0, 15, 60, and 300 mg/kg/day
Species/strain: rat, Sprague-Dawley (Slc:SD)(SPF)
Number/sex/group or time point (main study): 20
Route, formulation, volume, and infusion rate: oral (gavage) in 5 ml/kg 0.5 % aqueous methyl cellulose
Satellite groups used for toxicokinetics or recovery: 5/sex/group
Age: 5 weeks
Weight: 127.9-155.1 g (males) and 114.3-133.8 g (females)

Results

Mortality: One female in the control group, 4 males and 3 females in the 60 mg/kg/day group, and one male and 4 females in the 300 mg/kg/day group died after exhibiting crouching posture, gasping and pale auricles. At necropsy, dark reddish changes of the lungs (including bleeding) and foam in the trachea or rupture of the esophagus were observed, suggesting death from dosing error.

N = 20	Males (mg/kg/day)				Females (mg/kg/day)			
	0	15	60	300	0	15	60	300
Died or sacrificed moribund	0	0	4	1	1	0	3	4

Clinical signs: Significant clinical signs included ptosis, lacrimation, and salivation in all treated groups. Loose stools, diarrhea, deep respiration, decreased locomotor activity, and prone position were observed in the high dose group.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Clinical signs								
Eyelid ptosis	–	–	+	+	+	+	+	+
Lacrimation	–	–	+	+	+	+	+	+
Salivation	–	–	+	+	+	+	+	+
Stools loose	–	–	+	–	+	–	+	+
Diarrhea	–	–	–	–	–	–	+	+
Deep respiration	–	–	–	–	–	–	+	+
Decrease in locomotor activity	–	–	–	–	–	–	+	+
Prone position	–	–	–	–	–	–	–	+

Body weights: In males given 300 mg/kg, a tendency toward lower values was observed from Day 29 on, and significantly lower values were noted from Day 50 on. In males given 15 mg/kg, a tendency toward higher values was observed from Day 22 on, with significantly higher values in places. In females given 300 mg/kg, a tendency toward higher values was observed from Day 22 on, with significantly higher values in places.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Body weight (g)								
Day 50	389.0 ± 32.4	234.2 ± 17.2	414.0 ± 26.8	238.8 ± 15.6	394.2 ± 29.8	243.1 ± 25.7	362.0 ± 46.3*	260.9 ± 30.6*
Day 182	526.6 ± 49.4	297.0 ± 20.9	569.7 ± 52.9*	304.5 ± 18.6	526.9 ± 49.6	307.6 ± 35.7	465.4 ± 65.9**	319.8 ± 37.4

Food consumption: In males given 300 mg/kg, a tendency toward lower values was observed from Day 30 on. In males given 15 mg/kg, a tendency toward higher values was observed throughout the treatment period, with significantly different changes in places. In females given 300 mg/kg, significantly higher values were observed sporadically, but in general, food consumption in the female groups were similar to those of the control group throughout the treatment period.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Food consumption (g/day)								
Day 44	23.2 ± 2.1	16.9 ± 2.3	23.9 ± 2.4	16.3 ± 1.8	23.5 ± 2.4	17.0 ± 3.2	20.6 ± 2.8**	16.6 ± 2.7
Day 182	21.2 ± 2.5	14.5 ± 1.5	23.1 ± 2.6	14.5 ± 2.0	21.6 ± 2.4	15.4 ± 3.2	19.9 ± 2.9	15.4 ± 2.3

Ophthalmoscopy/audiology: No abnormalities were observed in the audiology test.

On macroscopic examination of the eyes, lacrimation, which was considered to be an incidental finding, was observed sporadically in females at the middle and end of the treatment periods. At the end of the treatment period, corneal opacity of the right eye, which was considered to be a spontaneous finding, was observed in 1 male control rat.

In the findings at the fundus photography, evidence of anemia was observed in 1 female given 60 mg/kg at the end of the treatment period and a linear corneal nebula, which was considered to be a spontaneous finding, was observed in 1 male control rat at the middle and end of the treatment periods. In the fundus photographs, incidental abnormal findings were observed sporadically in all treated and control groups, throughout the treatment period.

In some rats at each of the observation period, the fundus photographs were somewhat obscure, but no abnormalities were observed in these photographs.

Hematology: No treatment related effects were observed.

Clinical chemistry: In males given 300 mg/kg, higher values for total protein and albumin and lower values for triglycerides and non-esterified fatty acids were observed. In males given 15 mg/kg or more, higher values for blood glucose, potassium and inorganic phosphate were observed.

In females given 300 mg/kg, higher values for blood glucose and lower values for triglycerides were observed. In females given 15 mg/kg or more, higher values for potassium was observed.

In either sex given 300 mg/kg, higher values for cytochrome P-450 as either per unit microsomal protein or per unit liver weight were observed, as compared with the control groups.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Blood chemistry								
n=	20	19	20	20	16	17	19	16
Blood glucose (mg/dL)	163± 13	164± 11	174± 12*	165± 11	190± 14**	169± 12	183± 17**	177± 12**)
Potassium (mEq/L)	4.35± 0.40	4.51± 0.23	5.21± 0.56**	4.88± 0.45*	4.94± 0.37**	4.88± 0.60*	5.00± 0.44**	4.92± 0.64*
Inorganic phosphorus (mg/dL)	4.9± 0.4	4.8± 0.4	5.4± 0.5**	5.0± 0.4	5.4± 0.5**	5.1± 0.7	5.6± 0.3**	5.1± 0.5
Triglyceride (mg/dL)	184± 42	143± 50	188± 63	144± 75	161± 52	138± 98	65± 19**	75± 40**
Free fatty acid (mEq/L)	0. 39± 0.09	0.48± 0.15	0.48± 0.15	0.52± 0.14	0.41± 0.09	0.55± 0.15	0.28± 0.07**	0.51± 0.14
Albumin (g/dL)	2.5± 0.1	3.0± 0.2	2.5± 0.1	3.0± 0.3	2.6± 0.1	3.0± 0.2	2.7± 0.1**	3.0± 0.2
Total protein (g/dL)	6.2± 0.3	6.7± 0.3	6.3± 0.3	6.9± 0.5	6.4± 0.2	6.9± 0.4	6.6± 0.2**	6.8± 0.4

Urinalysis: At the middle of the treatment period, suspect positive (+) for occult blood was observed in 1 male given 300 mg/kg, in 1 male control rat, and in 2 males given 60 mg/kg. It was observed that a marked positive (3+) for occult blood in 1 female given 60 mg/kg and in 1 female given 15 mg/kg, and suspect positive (+) in 1 female given 15 mg/kg.

At the end of the treatment period, higher values for the urine volume were observed in males given 300 mg/kg. Red colored blood-like urine was observed in 1 female given 60 mg/kg. Marked positive (3+) for occult blood was observed in 2 females given 15 mg/kg.

In addition, findings without a dose-relation or with very slight changes were sporadically observed at the middle and end of treatment periods.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Urinalysis								
n=	20	19	20	20	17	17	19	16
Urinary volume (mL) at Week 26	9.9± 2.3	11.0± 3.9	11.1± 2.3	12.8± 6.8	11.8± 4.3	10.4± 4.0	14.8± 5.8**	11.2± 5.1

Gross pathology: In the liver, yellowish changes were observed in males given 60 mg/kg or more, and enlargement in males given 300 mg/kg. Depressed foci were observed in 1 male given 300 mg/kg. Whitish and dark reddish spots were observed in 1 male given 60 mg/kg, in 1 male given 15 mg/kg and in 1 male in the control group.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Autopsy findings								
n=	20	19	20	20	16	17	19	16
Liver: Yellow coloring or Yellowish white granular change	0	0	0	0	5	1	19	1
Enlargement	0	0	0	0	0	0	9	0

Organ weights:
In males given 60 mg/kg or more, higher adrenals weights were observed. In males given 300 mg/kg, higher liver weights were observed. Lower values in the absolute weights or higher values in the relative weights, due to the lower values for the final body weight were observed for the brain, thyroid, submandibular gland, heart, lungs, kidneys and prostate. In males given 15 mg/kg, higher values in the absolute weights or lower values

in the relative weights, due to the higher value in the final body weight, were observed for the heart, lungs, liver and pituitary.

In females given 300 mg/kg, higher weights for the liver and ovary, lower uterus weights, and higher values in the absolute weight of the adrenals were observed. In females given 60 mg/kg, higher values in the absolute weights of the kidney were considered to be incidental.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Organ weight								
n=	20	19	20	20	16	17	19	16
Absolute organ weight (g)								
Adrenal	0.058± 0.007	0.075± 0.007	0.059± 0.004	0.076± 0.010	0.066± 0.007**	0.076± 0.008	0.074± 0.011**	0.084± 0.009**
Liver	17.59± 2.35	10.03± 1.04	19.98± 2.91*	10.21± 1.03	19.02± 2.76	10.49± 1.37	21.84± 4.56**	11.56± 1.43**
Ovary		0.076± 0.013		0.074± 0.011		0.080± 0.011		0.090± 0.015**
Uterine		0.89± 0.28		0.87± 0.22		0.80± 0.28		0.51± 0.11**

Mean ± S.D., Dunnett's multiple comparison test (vs control): *; p<0.05 **; p<0.01.
a) 15 animals.

Dose (mg/kg/day)	0 (Control)		15		60		300	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Organ weight								
Relative organ weight (g/kg)								
Liver	33.35±2.20	33.75±2.43	34.93±2.36	33.38±2.53	36.04±2.89	34.14±2.20	46.89± 7.03**	36.16± 1.92**
Adrenal	0.110±0.012	0.251±0.026	0.104±0.008	0.248±0.032	0.126± 0.013*	0.251±0.031	0.161± 0.023**	0.264±0.030
Ovary		0.257±0.045		0.245±0.043		0.261±0.027		0.281±0.046
Uterine		3.01±0.93		2.86±0.70		2.59±0.74		1.62±0.35**

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

In the liver, findings included fatty degeneration of the hepatocytes in males given 15 mg/kg or more and in females given 60 mg/kg or more, swelling of the centrilobular hepatocytes in males given 15 mg/kg or more and in females given 300 mg/kg, and eosinophilic changes in the centrilobular hepatocytes in males given 60 mg/kg or more and in females given 300 mg/kg. Although granulomatous lesions were observed in all the groups, moderate to severe changes in such lesions were observed in males given 15 mg/kg or more and in females given 60 mg/kg or more. The severity and incidence of these findings were mostly dose-related and these findings were observed more frequently in males. Whitish and dark reddish spots and depressed foci in the macroscopic observation were necrotic foci of hepatocytes and focal fibrosis, respectively. Hyperplasia of the mammary grand was observed in females given 60 mg/kg or more, with lactation and atrophy of the uterus in females given 300 mg/kg. Hypertrophy of the epithelial cells of the vagina was observed in females given 60 mg/kg or more. The severity and incidence of these findings were dose-related. Hyperplasia of the mammary grand was observed in all male groups, and its incidence was higher in the

low-dose and control groups. Dilatation of the adrenal cortex was observed in males given 60 mg/kg or more.

	Males (mg/kg/day)				Females (mg/kg/day)			
	0	15	60	300	0	15	60	300
Fatty degeneration of hepatocytes								
__no remarkable change	18	3	0	0	19	20	16	13
__slight	2	16	10	0	0	0	1	3
__moderate	0	1	6	10	0	0	0	0
__severe	0	0	0	9	0	0	0	0
Swelling of centrilob.hepatocytes								
__no remarkable change	20	18	7	0	19	20	17	3
__slight	0	2	9	19	0	0	0	13
__moderate	0	0	0	0	0	0	0	0
__severe	0	0	0	0	0	0	0	0
Eosinophilic Δ of centrilob.hepat.								
__no remarkable change	20	20	18	0	19	20	17	14
__slight	0	0	3	19	0	0	0	2
__moderate	0	0	0	0	0	0	0	0
__severe	0	0	0	0	0	0	0	0
Granulomatous lesion								
__no remarkable change	2	0	1	0	0	2	0	0
__slight	18	19	11	10	14	18	13	15
__moderate	0	1	4	9	5	0	4	1
__severe	0	0	0	0	0	0	0	0
Adrenal gland, dilatat. of cortex								
__no remarkable change	20	20	14	5				
__slight	0	0	2	14				
__moderate	0	0	0	0				
__severe	0	0	0	0				
Uterus, atrophy								
__no remarkable change					19		17	8
__slight					0		0	8
__moderate					0		0	0
__severe					0		0	0
Vagina, hypertr of mucous epith.								
__no remarkable change					19	20	16	7
__slight					0	0	1	9
__moderate					0	0	0	0
__severe					0	0	0	0
Mammary gland, hyperplasia								
__no remarkable change					19	18	15	5
__slight					0	0	1	9
__moderate					0	0	0	1
__severe					0	0	0	0
Mammary gland, incr. secr. act.								
__no remarkable change					19	18	16	12
__slight					0	0	0	3
__moderate					0	0	0	0
__severe					0	0	0	0
Hepatic cyt P450 (nmol/mg prot.)	.34			.73**	.15			0.43**
Hepatic cyt P450 (nmol/g liver)	6.7			19**	3.1			9.0**

Electron microscopy:

In the liver, increase of lipid droplets in the hepatocytes was observed in males given 15 mg/kg or more and in females given 300 mg/kg. Proliferation of the smooth surfaced endoplasmic reticulum was observed in both sexes given 300 mg/kg.

No abnormal findings were observed in the kidneys.

Toxicokinetics: On Day 1 and in both Weeks 13 and 26, Cmax increased, and Tmax tended to prolong, relating to the doses.

Cmax in Weeks 13 and 26 tended to increase along with the time course, compared with that obtained in the initial dosing.

Plasma concentrations of KMD-3213 at 24 hr after dosing were detected in both sexes given 300 mg/kg during each treatment period, but showed no time-related tendency to increase. In both sexes given 60 mg/kg or less, the concentrations at 24 hr after dosing were less than or near to the detection limit.

In the control groups, plasma concentrations of KMD-3213 were lower than the detectable limit.

Dose (mg/kg/day)		0 (Control)		15		60		300	
N=		M: 20	F: 20	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Toxicokinetics:									
C_{max} (ng/mL)	Day 1	N.E.	N.E.	67.64± 29.42	91.92± 35.77	431.58± 152.11	519.23± 192.85	1421.50± 407.59	1106.38± 384.50
	Week 13	N.E.	N.E.	211.82 ± 83.11	232.63± 46.84	726.85± 230.89	705.81± 87.53	3587.83± 351.03	3004.5 7± 919.38
	Week 26	N.E.	N.E.	321.65± 94.80	192.29± 57.06	1443.37± 435.22	1098.13± 125.61	3810.00± 1968.25	3501.65± 670.20 a)
T_{max} (hr)	Day 1	N.E.	N.E.	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.3	5.4 ± 10.4
	Week 13	N.E.	N.E.	0.5 ± 0.0	0.5 ± 0.0	0.8 ± 0.3	0.7 ± 0.3	1.0 ± 0.0	1.0 ± 0.0
	Week 26	N.E.	N.E.	0.5 ± 0.0	0.6 ± 0.2	0.8 ± 0.3	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.3 a)

Mean ± S.D.
N.E.: Not Evaluated.
a) 4 animals.

Study title: Additional 26-week oral toxicity study of KMD-3213 in rats

Key study findings: At 0, 1, and 5 mg/kg/day, slight fatty degeneration of hepatocytes was observed, with a non significant increase at 5 mg/kg/day in males. No other effects were observed.

Study no.: Document No.: KMD-TX1999-204E02, Protocol No.: 10111
Conducting laboratory and location: Toxicology Research Laboratory, R&D Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 8 October 1997
GLP compliance: Japanese MHW
QA report: yes (x) no ( )
Drug, lot #, and % purity: lot no. JH312, 100.1% pure

Methods

Doses: 0, 1, and 5 mg/kg/day
Species/strain: rat, Sprague-Dawley (Slc:SD)(SPF)
Number/sex/group or time point (main study): 20
Route, formulation, volume, and infusion rate: oral (gavage) in 5 ml/kg 0.5% aqueous methyl cellulose
Satellite groups used for toxicokinetics or recovery: 5/sex/group
Age: 5 weeks
Weight: 131.9-162.8 g (males) and 104.8-143.5 g (females)
Sampling times:
Unique study design or methodology (if any):

Results

Mortality: No treatment related deaths occurred.

Clinical signs: Lacrimation was observed in the groups given 1 and 5 mg/kg. In males given 5 mg/kg, salivation was observed sporadically after 14 weeks.

Body weights: No treatment related effects were observed.

Food consumption: No treatment related effects were observed.

Ophthalmoscopy: No treatment related effects were observed.

Hematology: No treatment related effects were observed.

Clinical chemistry: No treatment related effects were observed.

Urinalysis: No treatment related effects were observed.

Dose (mg/kg/day)	0 (Control)		1		5	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Noticeable findings						
Number of animals died or sacrificed moribund	0	1 [b]	0	0	0	0
Body weights (g)	479.5 ± 44.5	285.1 ± 32.3	507.4 ± 48.9	294.8 ± 29.7	514.4 ± 43.8*	290.0 ± 31.3
Food consumption [a] (g/day)	20.6 ± 2.5	15.2 ± 2.4	21.6 ± 2.8	15.0 ± 2.3	22.0 ± 2.5	15.0 ± 2.9
Clinical signs						
Lacrimation	–	–	+	+	+	+
Salivation	–	–	–	–	+	–
Ophthalmology	–	–	–	–	–	–
Audiology	–	–	–	–	–	–
Hematology	–	–	–	–	–	–
Blood chemistry test	–	–	–	–	–	–
Urinalysis	–	–	–	–	–	–
Organ weight	–	–	–	–	–	–

Mean±S.D. Dunnett's multiple comparison test (vs control): * $p<0.05$.
–: No noticeable finding. +: Finding noted
a) At the end of treatment.
b) Death due to administration error.

Gross pathology: An accentuated lobular pattern in the liver was observed in 1 male given 5 mg/kg.

Histopathology:

Slight fatty degeneration of the hepatocytes was observed in 6 males and 1 female given 5 mg/kg, in 2 males given 1 mg/kg and in 2 males and 2 females of the control group. Slight hyperplasia of the mammary glands was observed in 1 female given 5 mg/kg.

Electron microscopy of the liver showed a slight increase in lipid droplets in the hepatocytes in one male given 5 mg/kg.

Dose (mg/kg/day)	0 (Control)		1		5	
N=	M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Necropsy findings						
n=	20	19	20	20	20	20
Liver: Clearer lobular pattern	0	0	0	0	1	0
Histopathology						
n=	20	19	20	20	20	20
Liver: Fatty degeneration of hepatocytes (mild)	2	2	2	Not done	6	1
Mammary gland: Hyperplasia (mild)	0	0 a)	Not done	Not done	0	1 a)
Electron microscopy						
n=	1	1	1	1	1	1
Liver: Increased lipid droplets in hepatocytes (mild)	–	–	–	Not done	+	–

-: No noticeable finding, +: Finding noted.
a) 18 animals.

Toxicokinetics: Cmax increased dose dependently. In the males given 5 mg/kg, Cmax was elevated with increased duration of dosing at Week 13 and Week 26, compared with at the initial dose. In the groups given 1 and 5 mg/kg, the plasma concentrations of test article at 24 hours after dosing were less than the detectable limit (N.D.) for each treatment stage.

Dose (mg/kg/day)		0 (Control)		1		5	
N=		M: 20	F: 20	M: 20	F: 20	M: 20	F: 20
Toxicokinetics							
Cmax (ng/mL)	Day 1	Not done	Not done	2.3 ± 0.2	2.5 ± 0.4	9.9 ± 4.9	9.5 ± 4.4
Week 13		Not done	Not done	1.0 ± 1.4	2.2 ± 0.3	22.9 ± 6.1	18.1 ± 7.3
Week 26		Not done	Not done	2.9 ± 0.2	2.4 ± 0.5	66.5 ± 59.2	21.0 ± 6.9
Tmax (hr)	Day 1	Not done	Not done	0.7 ± 0.3	0.5 ± 0.0	0.5 ± 0.0	0.6 ± 0.2
Week 13		Not done	Not done	0.5 a)	0.6 ± 0.2	0.5 ± 0.0	0.5 ± 0.0
Week 26		Not done	Not done	0.5 ± 0.0	0.8 ± 0.3	0.6 ± 0.2	0.5 ± 0.0

Mean ± S.D.
a) 2 animals.

Study title: Two-week intravenous toxicity study of MD127K in rats.

Key study findings: MD127K was found to be similar both in pharmacology and toxicology to KMD-3213.

Study no.: 10302 and 10308 (Plasma concentration analysis study for 2-week intravenous toxicity study of MD127K in rats.)

Conducting laboratory and location: Toxicology Research Laboratory, R&D, Kissei Pharmaceutical Co., Ltd.
Date of study initiation: 7 August 2003
GLP compliance: Japanese
QA report: yes (x) no ( )
Drug, lot #, and % purity: MD127K, Lot #056-021119-1, 99.0% pure and KMD-3213, lot #JZ011, 99.86% pure

Methods

Doses: 0, 2, 10, and 50 mg/kg/day of each compound
Species/strain: Slc:SD, SPF rat
Number/sex/group or time point (main study): 10
Route, formulation, volume, and infusion rate: intravenous, 0.1 M citric acid buffer
Age: 4-5 weeks
Weight: 141.0-167.0 g (males) and 115.8-146.3 g (females)

Results:

Mortality: No deaths occurred.

Clinical signs: week 1 (observed to a lesser extent in week 2)

MD127K (/ 70 observ.)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Deep respiration	0	4	10	32	0	3	10	24
Ptosis	0	16	26	45	0	7	12	32
Chromodacryorrhea	0	0	1	5	0	0	0	0
Lacrimation	0	0	2	1	0	0	1	2
Loose stool	0	0	0	0	0	0	0	0
Bloody feces	0	0	0	0	0	0	0	0
Dyspnea	0	0	0	0	0	0	0	1
Gasping	0	0	0	0	0	0	0	1

KMD-3213 (/ 70 observ.)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Deep respiration	0	2	8	27	0	3	7	25
Ptosis	0	24	39	50	0	10	33	43
Chromodacryorrhea	0	0	0	0	0	0	0	1
Lacrimation	0	0	1	0	0	0	1	1
Loose stool	0	0	0	3	0	0	0	2
Bloody feces	0	0	0	1	0	0	0	0
Continuous prone position	0	0	0	0	0	0	0	1
Decrease in locomotor act.	0	0	0	0	0	0	0	1

Body weights: No treatment related effects were observed for either KMD-3213 or MD127K.

Food consumption: No treatment related effects were observed for either KMD-3213 or MD127K.

Ophthalmoscopy: No treatment related effects were observed for either KMD-3213 or MD127K

Hematology: 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
MCHC (g/dl)	36.0	35.7	35.9	35.6*	37.0	36.8	37.0	36.8
Hb (g/dl)	15.6	15.2	15.6	15.8	15.8	15.7	16.0	16.3
Neutrophil (%)	12.8	16.2	13.3	12.7	11.4	11.8	10.5	12.0
Lymphocyte (%)	83.4	79.3	82.5	83.5	84.8	83.9	85.5	83.7
Monocyte (%)	1.4	1.8	1.5	1.3	1.2	1.5	1.4	1.3

KMD-3213 (N=10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
MCHC ((g/dl)	36.0	36.2	36.0	35.8	37.0	36.7	36.7	36.7
Hb (g/dl)	15.6	16.0	15.6	15.8	15.8	15.8	16.2	16.6*
Neutrophil (%)	12.8	12.6	12.7	13.1	11.4	13.4	13.0	15.5*
Lymphocyte (%)	83.4	83.5	83.4	83.2	84.8	82.9	82.9	79.9*
Monocyte (%)	1.4	1.4	1.4	1.7	1.2	1.4	1.4	1.8*

Clinical chemistry: 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
Albumin (%)	57.7	57.1	57.4	55.6*	58.9	55.0*	53.8**	55.9
Total protein (g/dl)	5.5	5.3	5.5	5.5	5.3	5.4	5.5*	5.5*
Calcium (mg/dl)	10.4	10.3	10.4	10.7	10.2	10.3	10.6*	10.4
α-globulin (%)	21.7	22.4	21.8	22.2	19.1	22.1	22.8**	22.1
Creatinine (mg/dl)	0.4	0.4	0.4	0.4*	0.4	0.4	0.4	0.4
Triglycerides (mg/dl)	106	113	93	103	78	59	69	65
Cl- (mEq/l)	105	106	106	106	108	109	107	107

KMD-3213 (N=10)	Males (mg/kg/day)				Females (mg/kg/day)			
	0	2	10	50	0	2	10	50
Albumin (%)	57.7	57.1	57.5	55.3	58.9	55.4*	55.7	54.9*
Total protein (g/dl)	5.5	5.4	5.3	5.3	5.3	5.6**	5.7**	5.4
Calcium (mg/dl)	10.4	10.4	10.6	10.4	10.2	10.5	10.6	10.2
α-globulin (%)	21.7	22.2	21.8	23.4	19.1	21.7*	22.4**	22.6**
Creatinine (mg/dl)	0.4	0.4	0.4*	0.4	0.4	0.4	0.4	0.4
Triglycerides (mg/dl)	106	101	111	100	78	62	56	44*
Cl- (mEq/l)	105	106	107*	106	108	107	107	107

Urinalysis: 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
Specific gravity (g/ml)	1.058	1.059	1.063	1.057	1.065	1.068	1.068	1.067
Cl- (mEq/l)	179	178	186	179	205	217	199	184
Urine volume (ml/day)	10.5	10.0	8.7	11.5	6.1	8.3	7.7	7.1
K+ (mEq/l)	370.4	378.1	383.8	341.2	429.1	434.6	399.8	398.8