CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:
22-023

PHARMACOLOGY REVIEW

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:	22-023
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	March 31, 2006, Electronic Submission
PRODUCT:	Fosaprepitant (Emend I.V) Injection
INTENDED CLINICAL POPULATION:	Chemotherapy-induced nausea and vomiting (CINV)
SPONSOR:	Merck & Co., Inc., West point, PA.
DOCUMENTS REVIEWED:	Electronic submission of the NDA
REVIEW DIVISION:	Division of Gastroenterology Products (HFD-180)
PHARM/TOX REVIEWER:	Sushanta Chakder, Ph.D.
PHARM/TOX SUPERVISOR:	Jasti B. Choudary, B.V. Sc., Ph.D.
DIVISION DIRECTOR:	Brian Harvey, M.D., Ph.D.
PROJECT MANAGER:	Giuseppe Randazzo

Date of review submission to Division File System (DFS): January 17, 2007

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EXECUTIVE SUMMARY

I. Recommendations

Recommendation on approvability: The sponsor conducted adequate preclinical studies with fosaprepitant/aprepitant to determine the safety of the drug. The toxicity of the drug has been adequately characterized for its use at the intended dose and route of administration. Thus, from a preclinical standpoint, the NDA application is approvable.

A. **Recommendation for nonclinical studies:** None.

B. **Recommendations on labeling:** Included in the labeling section of the review.

II. Summary of nonclinical findings

A. Pharmacology:

Substance P, a sensory tachykinin, may have an important role in emesis, as substance P-containing vagal afferent fibers innervate the brainstem nucleus tactus solitarius, a region of the CNS involved in emesis. Fosaprepitant is a precursor of aprepitant, and is rapidly converted to aprepitant following i.v. administration to animals and humans. After i.v. dosing of fosaprepitant to rhesus monkeys, a good correlation between plasma aprepitant concentration and central receptor occupancy was observed. Aprepitant caused an inhibition of binding of [^{125}I]substance P to cloned human, rat, dog and guinea pig NK_1 receptors expressed in cultured cells, and to NK_1 receptors from dog, monkey, mouse, rabbit and ferret cerebral cortex with IC_{50} values in the nM range. Oral or i.v. aprepitant caused dose-dependent increases in the latency of cisplatin-induced retching and vomiting and reduced the number of retches and vomits in ferrets. An additive anti-emetic effect was observed when the drug was administered with dexamethasone. At suboptimal doses, dexamethasone (20 mg/kg i.v.) or MK-0869 (0.1 mg/kg i.v.) was not effective in preventing cisplatin-induced emesis in ferrets, but the combination caused a significant inhibition of emesis in this animal model.

B. ADME:

Pharmacokinetics/toxicokinetics studies were conducted with both fosaprepitant (L-758, 298) and the active drug (aprepitant; L-754, 030/MK-0869) following i.v. and oral administration to rats, mice, dogs and ferrets. Fosaprepitant (L-758,298) was rapidly converted to aprepitant in *in vitro* liver fractions from rat, dog and human and also by the S9 fractions from human lung, kidney and ileum, suggesting that the conversion of the pro-drug to active drug can take place in extrahepatic tissues. The terminal half-lives of intravenous aprepitant in rats and ferrets were approximately 3 and 10 hours, respectively. In humans, the apparent terminal half-life of aprepitant ranged from approximately 9 to 13 hours. Aprepitant penetrated the blood brain barrier in rats and ferrets following i.v. and oral administration, and excreted in the milk of lactating rats. In human liver microsomes, CYP3A4 was the major CYP enzyme involved in the metabolism of MK-0869. Two other CYP enzymes, CYP1A2 and CYP2C19 were also involved in the metabolism of the compound. Fosaprepitant caused a moderate induction of cytochrome P_{450} enzyme activity (7-ethoxy-4-trifluoromethylcoumarin-O-deethylase and fatty acyl-CoA oxidase activity) in the mouse liver. It also caused a moderate inhibition of CYP3A4. The liver enzyme inducing capability of the drug may be responsible for the hepatocellular hypertrophy and thyroid follicular cell hyperplasia observed in rats during repeated administration of the drug.

C. Toxicology:

Acute toxicity studies of fosaprepitant (L-758, 298) were conducted in mice and rats following i.v. administration of 200 and 500 mg/kg doses, and following an oral dose of 500 mg/kg. The minimal lethal dose (MLD) by the i.v. route was 500 mg/kg in both rats and mice. There were no deaths of rats and mice at the 500 mg/kg oral dose. The clinical signs observed in mice after i.v. dosing included gasping, convulsions, bradypnea and loss of righting reflex, which disappeared within 3 hours. In rats, the clinical signs included gasping and bradypnea.

Repeat dose toxicity studies with aprepitant (MK-0869) were conducted in rats, mice, dogs and monkeys. Seventeen-day i.v. toxicity studies with the lyophilized i.v. formulation of fosaprepitant were conducted in rats and dogs. In rats, fosaprepitant doses of 2.5, 5.0 and 7.5 mg/kg/day were used, and injection site changes (cellular proliferation of venous intima, venous necrosis or thrombosis, skin necrosis, subcutaneous edema, cellular infiltration and degeneration of muscle fibers) were observed in all groups. In the 17-day i.v. toxicity study in dogs, the injection site was also the target organ of toxicity (venous thrombosis, fibroplasia and necrosis), and the 2.0 mg/kg/day dose was the no effect dose. In repeat dose oral toxicity studies with aprepitant in rats, the target organs of toxicity were the liver (centrilobular hepatocellular hypertrophy), pituitary (vacuolation) and the thyroid gland (follicular cell hyperplasia), and these effects may be related to the activation of hepatic drug metabolizing enzymes. In a 5-week i.v. toxicity study with L-758, 298 in dogs, the no effect dose was 2 mg/kg/day, and no target organs of toxicity were identified. In a 39-week oral toxicity study with aprepitant in dogs, the target organs of toxicity were the testes (tubular degeneration) and prostate (atrophy). Testicular degeneration and an atrophy of the prostate and thymus was also observed in a 5-week oral toxicity study in dogs. However, in a 53-week oral toxicity study with a 27-week interim sacrifice, no target organ of toxicity was identified. In monkeys, intravenous dosing of L-758, 298 for up to 240 mg/kg/day for 17 days, and up to 10 mg/kg/day for 5 weeks was not associated with any adverse effects, and no target organs of toxicity were identified. Thus, repeated intravenous administration of fosaprepitant/aprepitant in rodents and non-rodents was not associated with any toxic effects other than the injection site reactions.

The genotoxic potential for L-758, 298 (fosaprepitant) was examined in the bacterial reverse mutation assay (Ames assay), the rat hepatocyte DNA damage assay, the mutagenicity assay in TK6 human lymphoblastoid cells, the chromosomal aberrations assay in the Chinese hamster ovary cells, and the *in vivo* mouse bone marrow micronucleus assay. L-758, 298 was genotoxic in any of the assays.

The genotoxic potential for L-754, 030 (aprepitant; MK-0869) was examined in the bacterial reverse mutation assay (Ames assay), the *in vivo* mouse bone marrow micronucleus assay and the mutagenesis assay in TK6 human lymphoblastoid cells. It had no genotoxic potential in any of these assays.

The sponsor conducted three 2-year oral carcinogenicity studies in Sprague-Dawley rats and two 2-year oral carcinogenicity studies in mice with aprepitant (MK-0869). In the rat carcinogenicity studies, animals were treated with oral doses ranging from 0.05 to 1000 mg/kg twice daily. The highest dose produced systemic exposure to aprepitant (plasma $AUC_{0\text{-}24\,hr}$) of 0.7

to 1.6 times the human exposure ($AUC_{0\text{-}24\,hr}$ = 19.6 mcg.hr/ml) at the recommended oral dose of 125 mg/day. Treatment with aprepitant at a dose of 1000 mg/kg twice daily caused an increase in thyroid parafollicular cell carcinoma, and at doses of 5 to 1000 mg/kg twice daily it caused an increase in the incidences of thyroid follicular cell adenomas and carcinomas in male rats. In female rats, it produced hepatocellular adenomas at 5 to 1000 mg twice daily and hepatocellular carcinomas and thyroid follicular adenomas at 125 to 1000 mg/kg twice daily. In the mouse carcinogenicity studies, the animals were treated with oral doses ranging from 2.5 to 2000 mg/kg/day. The highest dose produced a systemic exposure of about 2.8 to 3.6 times the human exposure at the recommended dose. Treatment with aprepitant produced skin fibrosarcomas at 125 and 500 mg/kg/day doses in male mice.

Reproductive and developmental toxicology studies were conducted with both fosaprepitant (pro-drug; L-758,298) and aprepitant (active drug; L-754,030; MK-0869) by the intravenous and oral routes of administration. In the i.v. Segment I fertility and general reproductive performance study in rats, fosaprepitant had no treatment-related effects on the fertility and general reproductive performance of male and female rats at i.v. doses up to 10 and 4 mg/kg/day, respectively. MK-0869 had no effects on the fertility and general reproductive performance of male and female rats at oral doses up to 2000 mg/kg/day. L-758, 298 was not teratogenic in rats and rabbits at i.v. doses up to 4 mg/kg/day. Fosaprepitant had no effects on the peri- and post- natal development in rats at i.v. doses up to 4 mg/kg.

B. **Nonclinical safety issues relevant to clinical use:** Toxicology studies conducted with fosaprepitant or aprepitant did not reveal any nonclinical safety issues relevant to clinical use of the drug.

PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-023

Review number: 01

Sequence number/date/type of submission: 000/March 31, 2006/Original (Electronic submission).

Information to sponsor: Yes ( ) No (X)

Sponsor and/or agent: Merck & CO., Inc., West Point, PA.

Manufacturer for drug substance: Merck Research Laboratories, Whitehouse Station, NJ 08889.

Reviewer name: Sushanta Chakder, Ph.D.

Division name: Division of Gastroenterology Products

HFD #: 180

Review completion date:

Drug:

Trade name: Emend I.V.

Generic name: Fosaprepitant dimeglumine

Code name: MK-0517/L-000758298

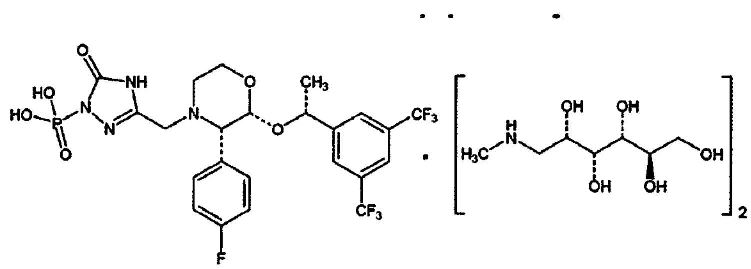
Chemical name: 5-[[(2R, 3S)-2-[(1R)-1-[3, 5-bis(trifluoromethyl) phenyl] ethoxy]-3-(4-fluorophenyl)-4-morpholinyl] methyl]-1, 2-dihydro-3H-1, 2, 4-triazol-3-one.

CAS registry number: 265121-04-8

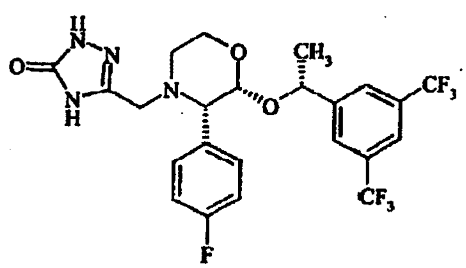
Molecular weight: 1004.83 Da

Structure:

Fosaprepitant



Aprepitant



Relevant INDs/NDAs/DMFs:

IND 48, 924, Merck & Co., Inc., West Point, PA.

IND 50, 283, Merck & Co., Inc., West Point, PA.

NDA 21-549, Emend Tablets, Merck & Co., Inc., West Point, PA.

Drug class: Neurokinin 1 (NK_1)/Substance P receptor antagonist.

Indication: In combination with other antiemetic agents, for the prevention of acute and delayed nausea and vomiting associated with initial and repeat courses of highly emetogenic cancer chemotherapy.

Clinical formulation: Each 10 ml vial contains fosaprepitant dimeglumine (115 mg fosaprepitant ——— and the following excipients (mg/ml): edetate disodium ——, polysorbate 80 ——, lactose anhydrous ——, sodium hydroxide (c ———) and hydrochloric acid ——— b(4)

Route of administration: Intravenous

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

STUDY	STUDY #	LOT #	TESTING LAB	PAGE # OF REVIEW
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Absorption				
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Pharmacokinetics and oral bioavailability of MK-0869 in male CD-1 mice	--	--	--	53
Pharmacokinetics and oral bioavailability of MK-0869 in male ferrets	--	--	--	55
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Pharmacokinetics of L-758298 in male and female dogs using the new higher concentration formulation	--	--	--	58
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Tissue distribution and excretion of radioactivity Following single i.v. dose of [^{14}C]L-758298 in Male rats	--	--	--	60
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Plasma protein binding of L-754,030 in plasma from rat, dog and human	40001359	--	--	67
Pharmacokinetics and brain/plasma distribution of MK-0869 in CD-1 mice	--	--	--	68
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b(4)

b(4)

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Oral toxicokinetic study in pregnant rats	01-732-0	--	--	101
Oral toxicokinetic study in pregnant and lactating Rats	01-738-0	--	--	102
I.V. toxicokinetic study in pregnant and lactating Rats	97-741-0	--	--	104
I.V. toxicokinetic study with L-758,298 in pregnant rats	98-708-0	--	--	104
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L-758,298: Oral and i.v. toxicity studies in mice and rats	95-2584, -2586, -2587	--	Merck Research Laboratories, West Point, PA.	107
L-754030: Acute oral and I.P. toxicity studies in Mice and rats	95-2685, -2688,-2689	#8	Merck Research Laboratories, West Point, PA.	108
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L-000758298: 17-day i.v. toxicity study in rats	04-6005	003C016	Merck Research Laboratories, Cedex 9, France	110
MK-0869: 16-day i.v. toxicity study in rats	99-004-0	004H032	Merck Sharp & Dohme, Riom, France.	116
4-week i.v. toxicity study of L-758298 in rats	95-607-0	--	Merck Research Laboratories, West Point, PA.	118
L-758298: 5-week i.v. toxicity study in rats	97-002-0	003C009	Merck Research Laboratories, West Point, PA.	121
L-754030: 5-week oral range finding and toxicokinetic study in rats	97-060-0	#21	Merck Research Laboratories, West Point, PA.	124
MK-0869: 5-week oral toxicity study in rats	00-001-0,-1	004H031	Merck Research Laboratories, West Point, PA.	128
L-754030: 14-week oral range-finding study in rats	96-051-0	004H002, 004H004	Merck Research Laboratories, West Point, PA.	136
L-754030: 14-week oral range-finding study in rats	97-117-0	004H026	Merck Research Laboratories, West Point, PA.	140
L-754030: 14-week oral toxicity study in rats	95-627-0	L-754030	Merck Research Laboratories,	144

		-000Z	Cedex 9, France.	
MK-0869: 27-week oral toxicity study in rats	01-092-0	016S001	Merck Research Laboratories, West Point, PA.	147
L-754,030: 53-week oral toxicity study in rats with 27-week interim necropsy	97-071-0	004H021, 004H026	Merck Research Laboratories, West Point, PA.	153
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L-000758298: 17-day i.v. toxicity study in dogs	04-6004	010A001	Merck Research Laboratories, Cedex 9, France.	158
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53-week oral toxicity study in dogs with a 27-week interim necropsy	97-614-0	#21	Laboratoires Merck-Sharp & Dohme-Chibret, Riom, France	197
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L-754030-Microbial mutagenesis assay	95-8043, 95-8045	000Z008	Merck Research Laboratories, West Point, PA.	207
DNA damage in rat hepatocytes	95-8413	--	Merck Research Laboratories, West Point, PA.	210
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Chromosome aberration in CHO cells	95-8669, 95-8672	000Z008	Merck Research Laboratories, West Point, PA.	212

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Mouse bone marrow micronucleus assay	98-8608	004H030	Merck Research Laboratories, West Point, PA.	219
TK6 Human cell mutagenesis assay with L-758298	98-8308	004H030	Merck Research Laboratories, West Point, PA.	220
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2-Year mouse carcinogenicity studies	--	--	Merck Research Laboratories, West Point, PA.	227
Reproductive and Developmental Toxicology:				
Segment I (i.v.) fertility and reproductive performance study of L-758,298 in female rats	96-714-0	--	Merck Research Laboratories, West Point, PA.	229
Oral fertility study of MK-0869 in female rats	01-735-0	X0869OPP 024C001	Merck Research Laboratories, West Point, PA.	233
Oral fertility study in male rats	97-734-0	004H021	Merck Research Laboratories, West Point, PA.	235
Oral fertility study in male rats	01-737-0	X0869OPP 024C001	Merck Research Laboratories, West Point, PA.	237
I.V. fertility study with L-758298 in male rats	98-704-0	003C013	Merck Research Laboratories, West Point, PA.	240
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L-754,030: Oral developmental toxicity study in rabbits	96-716-0	000Z010	Merck Research Laboratories, West Point, PA.	247
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L-000758298: Exploratory 5-day i.v. tolerability study in rats	03-2622	--	Merck Research Laboratories, West Point, PA.	262
L-000758298: Exploratory 5-day i.v. tolerability Study in female rats	04-0228	--	Merck Research Laboratories, West Point, PA.	264
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5-Week oral thyroxine clearance study in rats 5-day i.v. range-finding toxicity study in the rat	98-153-0	004H026	Merck Research Laboratories, West Point, PA.	270
In vitro hemolysis assay in washed red blood cells and whole blood	94-4905	--	Merck Research Laboratories, West Point, PA.	272
Exploratory enzyme induction studies in mice	94-261-0,-1	--	Merck Research Laboratories, West Point, PA.	273
Enzyme induction by L-758,298 in mice	94-258-0,-4	--	Merck Research Laboratories, West Point, PA.	275
L-754,030: Exploratory 16-day oral hepato-Toxicity study in rats	96-613-0	--	Merck Research Laboratories, West Point, PA.	276

Studies not reviewed within this submission: None

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Substance P, a sensory tachykinin, may have an important role in emesis, as substance P-containing vagal afferent fibers innervate the brainstem nucleus tactus solitarius, a region of the CNS involved in emesis. Fosaprepitant is a precursor of aprepitant which is a selective and potent neurokinin$_1$ (NK_1) receptor antagonist, as shown by its affinities for cloned and native NK_1 receptors from different species. The antiemetic effect of aprepitant has been assessed, either alone or in combination, in different animal models. It has been found to be very effective in preventing both the initial and delayed phases of cisplatin-induced emesis in ferrets. It showed additive effects on the anti-emetic effects of ondansetron or dexamethasone in this animal model. Fosaprepitant, when administered intravenously to animals and humans, is very rapidly converted to aprepitant. Thus, intravenous fosaprepitant has the potential for preventing chemotherapy-induced emesis in cancer patients.

2.6.2.2 Primary pharmacodynamics

Receptor Binding:

<u>IC_{50}s (nM) of L-754,030 and L-758,298 for $[^{125}I]$-Tyr^8-substance P at NK_1 receptors from rat, dog, guinea pig, ferret and human</u>

Human NK_1 receptors were cloned, expressed in Chinese hamster ovary (CHO) cells and incubated with $[^{125}I]$-Tyr^8-substance P. Cells were harvested by filtration, radioactivity was determined with scintillation counters, and data were analyzed with the —— program —— Studies of human NK_1 receptors were conducted in the presence or absence of 1% human serum albumin. Rat NK_1 receptors were studied in a manner similar to that for human NK_1 receptors. Guinea pig NK_1 receptors were expressed in COS cells instead of CHO cells. On the other hand, the interactions of L-754,030 and L-758,298 with $[^{125}I]$-Tyr^8-substance P at dog and ferret NK_1 receptors were studied in cortical synaptosomal membrane preparations. b(4)

As shown in the following table, IC_{50}s were lower for L-754,030 than L-758,298 in all species, indicating higher affinities for L-754,030. IC_{50}s were in the nanomolar range for all species and, thus, differences among species probably are not biologically significant. In the case of human NK_1 receptors in the presence of 1% human serum albumin, IC_{50}s for L-758,298 and L-754,030 were 30 nM and 0.3 nM, respectively. Again, the difference in IC_{50}s in the presence and absence of 1% human serum albumin is probably not biologically significant.

Thus, the IC_{50}s in the nanomolar range suggest relatively potent affinities of L-758,298 and 754,030 for NK_1 receptors in all species that were studied.

IC_{50}s (nM) of L-758,298 and L-754,030 for [^{125}I]substance P at NK_1 receptors from rat, dog, guinea pig, ferret and human

Source of NK_1 receptors	IC_{50} (nM) of L-758,298 and L-754,030 for [^{125}I]substance P	
	L-758,298	L-754,030
Rat (unspecified)	33	3.7
Dog Cortex	0.9	0.4
Guinea Pig (unspecified)	7	0.1
Ferret Cortex	1.1	0.7
Human (unspecified)	3.3	0.1

Determination of the Affinity of L-754, 030 for the Human, Guinea Pig, Ferret, Dog and Rat NK_1 Receptors in the Presence or Absence of Human Serum Albumin.

Methods: The binding affinities of L-754030 for cloned human NK_1 receptors, expressed in Chinese hamster ovary (CHO) cells, were evaluated in the presence or absence of 1% human serum albumin. The binding affinities for human receptors were compared with that of guinea pig, ferret, dog and rat NK_1 receptors. The CHO cells expressing the receptors were incubated with [^{125}I]-Tyr-Substance P (2000 Ci/mmol) and the compound at room temperature for <40 minutes, and the receptor ligand complex was separated by filtration. Similar assays were conducted with CHO cells expressing rat

NK_1 receptors, or in COS cells expressing the cloned guinea pig NK_1 receptors. NK_1 receptor affinities for dogs and ferrets were determined using synaptosomal membranes purified from the cortex.

Results: L-754030 caused an inhibition of [^{125}I]-substance P binding to human NK_1 receptors with an IC_{50} of 0.1±0.07 nM and a Kd of 86±20 pM. In the presence of 1% human serum albumin, the IC_{50} for inhibition of radiolabeled substance P binding to the human NK_1 receptors was 0.3 nM. L-754030 had the same affinities for the human and guinea pig NK_1 receptors (0.1 nM for both) while the affinities for ferret (0.7 nM), dog (0.4 nM) and rat (3.7 nM) receptors were 7-, 4- and 37-fold lower, respectively.

IC_{50}s of L-754,030 and L-758,298 for cis-(+)-[n-methyl-^{3}H]-diltiazem at the diltiazem binding site on the L-type calcium channel from rabbit skeletal muscle

Rabbit skeletal muscle membranes were prepared from hindleg muscle. Binding on the L-type calcium channel in the skeletal muscle membranes was assessed by using cis-(+)-[n-methyl-^{3}H]-diltiazem (NEN, specific activity of 60-87 Ci/mmol) which binds to a site in the channel. Twenty-five µl of cis-(+)-[n-methyl-^{3}H]-diltiazem (final concentration of 10 nM) was added to 0.1 ml of the membrane preparation (50-75 µg/assay or 200 mg/ml tissue wet weight) in a final volume of 0.5 ml using 50 mM Tris, pH 7.4. Test compounds (L-754,030 and L-758,298) were added in 4-5 µl of DMSO. Radioactivity was quantitated in scintillation counters.

The IC_{50}s of L-754,030 and L-758,298 for cis-(+)-[n-methyl-^{3}H]-diltiazem were 8 and 6 µM, respectively. The IC_{50}s in the micromolar range suggest relatively weak affinities of L-754,030 and L-758,298 for diltiazem receptors.

Determination of the Binding Affinities of MK-0869 for the Rhesus Monkey, Mouse and New Zealand White Rabbit NK_1 Receptors

Brain synaptosomal membrane preparations from Rhesus monkey, mouse or New Zealand white rabbit brain were used to determine the binding affinities of MK-0869 for NK_1 receptors. The inhibition of binding of ^{125}I-Tyr^8-Substamce P to the synaptosomal membrane preparations by different concentrations of MK-0869 was determined following incubation under equilibrium conditions.

MK-0869 had similar binding affinities to the native NK_1 receptors from the Rhesus monkeys and rabbits, while it had >39-fold reduced affinity for the native mouse NK_1 receptor. The reduced affinity of MK-0869 for the mouse NK_1 receptor was similar to that observed with the cloned rat NK_1 receptor (46-fold reduction), as reported previously. The binding affinities of MK-0869 for the monkey, mouse and rabbit NK_1 receptors are shown in the Table below.

Comparison of the Binding Affinities of MK-0869 for Rhesus Monkey, Mouse and New Zealand White Rabbit NK_1 Receptors

NK_1 Receptor Species	IC_{50} (nM)
Rhesus Monkey	0.090 ± 0.008 (2)
Mouse	4.7 ± 0.1 (2)
New Zealand White Rabbit	0.091 ± 0.013 (2)

Data represent mean IC_{50} ± SD (number of determinations)

Determination of Binding Affinities of Fosaprepitant for Human, Ferret, Rat, Dog and Guinea Pig NK_1 Receptors.

The binding affinities of fosaprepitant (L-000758298; MK-0517) in cloned human, rat, guinea pig NK_1 receptors, and in NK1 receptors from ferret and dog synaptosomal preparations were determined by *in vitro* receptor binding assay. Human and rat NK_1 receptors were cloned and expressed in Chinese hamster ovary (CHO) cells, and the guinea pig receptors were expressed in COS-7 cells. Brain synaptosomal preparations containing native NK_1 receptors were prepared from ferret and dog brain cortex. Displacement of ^{125}I-Tyr^8-Substance P binding by different concentrations of fosaprepitant was determined following incubation at room temperature.

Fosaprepitant showed high affinities for human NK_1 receptors with an IC_{50} of 2.1±2.1 nM. The affinity of fosaprepitant for the human NK_1 receptors was reduced 14-fold (IC_{50}, 30 nM) with the inclusion of human serum albumin, suggesting fosaprepitant has a low affinity for serum albumin. As compared to human receptors, fosaprepitant had 3.6-fold and 11-fold lower affinities for recombinant guinea pig and rat NK_1 receptors, respectively, and had 1.9-fold and 2.3-fold higher affinities for the native ferret and dog NK_1 receptors, respectively. The binding affinities of fosaprepitant for the NK_1 receptors from different species are shown in the Table below.

Table 1. Determination of the Binding Affinities of Fosaprepitant for the Human, Guinea Pig, Ferret, Dog and Rat NK_1 Receptors

NK_1 Receptor Species	IC_{50} as nM (n)
Human	2.1 +/- 2.1 (14)[a]
Guinea pig	7.5 +/- 2.3 (2)
Ferret	1.1 (1)
Dog	0.9 (1)
Rat	24 (1)

[a] Data represent mean IC_{50} +/- S.D.

Binding selectivity of L-758,298 and L-754,030 for the human NK_1 receptor versus other human G-protein coupled receptors

The inhibition of unspecified agonists by L-758,298 and L-754,030 (both at 1 μM and 10 μM concentrations) at human NK_2; NK_3; adrenergic α_{1a}, α_{1b}, α_{1c}, α_{2a}, α_{2b}, α_{2c}, β_1, β_2 and β_3; muscarinic M_1, M_2, M_3, M_4 and M_5; dopaminergic D_2, D_3 and D_4; C_{5a}; IL-8, endothelin A; endothelin B; glucagon; GLP-1; NPY-Y_1 and NPY-Y_2 receptors were assessed. Procedural details were not provided.

The sponsor provided % inhibition of unspecified agonists by each concentration of L-758,298 and L-754,030 at each receptor; sponsor did not provide IC_{50} estimates.

According to the sponsor, L-754,030 is 3,000-fold selective for the human NK_1 receptor versus the human NK_3 receptor and >50,000-fold selective versus the other G-protein coupled receptors that were studied.

According to the sponsor, L-758,298 is >3,000-fold selective for the human NK_1 receptor versus the human NK_3 receptor, 818-fold selective versus the endothelin A receptor, and >3,000-fold selective versus the other receptors studied.

Binding selectivity of L-754,030 for receptors and enzymes from mouse, rat, guinea pig, pig, hamster, calf and man

The inhibition of specified ligands by L-754,030 (0.01, 0.1, 1 and 10 μM concentrations) at 90 different receptors and enzymes from mouse, rat, guinea pig, pig, hamster, calf and man was assessed. Procedural details were not provided.

The sponsor provided % inhibition of each agonist by each concentration of L-754,030 at each receptor; sponsor did not estimate IC_{50}s.

The only receptor at which L-754,030 was inhibitory in the micromolar range was the NK_1 receptor from the rat submaxillary gland; the ligand was [^{3}H]-substance P. The inhibition by L-754,030 was 93, 58, 21 and 8% at the 10, 1, 0.1 and 0.001 μM concentrations, respectively. Inhibition of binding by L-754,030 at all other sites studied was weaker than that for the NK_1 receptor.

Binding kinetics of [^{125}I]-Tyr^8-substance P and [^{3}H]-L-754,030 for human NK_1 receptors

Human NK_1 receptors were cloned, expressed in CHO cells and incubated with [^{125}I]-Tyr^8-substance P. The binding of [^{125}I]-Tyr^8-substance P (91 pM to 1.5 nM) to human NK_1 receptors was assessed by plotting dissociation curves and deriving Scatchard plots (bound substance P/free substance P plotted as a function of bound substance P); K_ds (estimates of affinity) and B_{max}s (estimates of number of binding sites) were estimated from the Scatchard plots. Scatchard analysis revealed a K_d of 41 pM and B_{max} of 170 fmol/mg protein for [^{125}I]-Tyr^8-substance P.

When the interactive effects of unlabeled L-754,030 and [^{125}I]-Tyr^8-substance P (91 pM to 1.5 nM) were studied in human NK_1 receptors, K_d of [^{125}I]-Tyr^8-substance P was decreased, and B_{max} of [^{125}I]-Tyr^8-substance P was decreased. These data suggest L-754,030 may be a noncompetitive antagonist at the NK_1 receptor.

In another experiment, binding of [^{3}H]-L-754,030 to human NK_1 receptors expressed in baculovirus-infected Sf9 cell was assessed. K_d was 0.2 nM; B_{max} was not provided by the sponsor. Thus, the affinity of L-754,030 for human NK_1 receptors is less than that for [^{125}I]-Tyr^8-substance P.

Inhibition of the binding of [^{125}I]-L-703,606 to human NK_1 receptors by L-754,030

The binding properties of L-703,606 to human NK_1 receptors have been previously characterized by the sponsor. L-703,606 was found to be a competitive antagonist at the NK_1 receptor with a K_d of 0.5 nM. Thus, the sponsor investigated the effect of L-754,030 on the binding of [^{125}I]-L-703,606 to the human NK_1 receptor to verify that L-754,030 is interacting at the same receptor site. Thus, human NK_1 receptors were cloned, expressed in CHO cells and incubated with [^{125}I]-L-703,606. L-754,030 inhibited the binding of [^{125}I]-L-703,606 to the human NK_1 receptors with an IC_{50} of 0.2 nM and, therefore, is most likely binding at the same site as [^{125}I]-L-703,606.

Evaluation of the Binding Affinities of *In Vitro* Metabolites and Derivatives of MK-0869 for the Human NK_1 Receptor.

Methods: The binding affinities of several *in vitro* metabolites and derivatives of MK-0869 were determined at cloned human NK_1 receptors expressed in Chinese Hamster Ovary (CHO) cells. The metabolites and derivatives used in the study were L-755446, L-829674, L-825678 and L-324261, L-846188, L-872945, L-384971, L-863664 and L-764120. All the metabolites, except L-858442 and L-858443, were detected in the systemic circulation of rats and humans.

Results: Four metabolites of MK-0869 (L-755446, L-829674, L-825678 and L-324261) had high binding affinities for cloned human NK_1 receptors with affinities of 3.6 nM or lower (IC_{50}). Other metabolites had greatly reduced ($IC_{50} \geq 240$ nM) or moderate (IC_{50} 10-30 nM) binding affinities. Overall, the metabolites had 2.7 to 35-fold lower affinities for human NK_1 receptors as cpmpared with the parent compound, MK-0869.

Binding Activity of MK-0869 and Selected Metabolites at Human $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$ Receptors, Human 5-HT, NE and DA Transporters and Rat Vesicular Transporter.

Methods: The selectivity of binding of MK-0869 and its metabolites, L-755446, L-809861, L-809771, L-825678, L-829615 and L-829617 for human $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$ receptors, the human 5-HT, NE and DA transporters and rat vesicular transporters were examined by their binding activity at human cloned $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$ receptors and at cloned human 5-HT, NE and DA transporters and at rat monoamine vesicular transporters.

Results: MK-0869 and its metabolites showed minimal binding affinity at the cloned human $5\text{-}HT_{1A}$, $5\text{-}HT_{2A}$, $5\text{-}HT_{2C}$ receptors and at cloned human 5-HT, NE and DA transporters and at rat monoamine vesicular transporters with IC_{50} values ranging from >3000 nM to >10, 000 nM.

Inhibition of substance P-induced inositol phosphate synthesis by L-754,030

NK_1 receptors are coupled to an inositol phosphate second messenger system. Inositol triphosphate (IP_3) functions as a second messenger by stimulating the release of Ca^{2+} from intracellular stores. When IP_3 is actively serving as a second

messenger, there is IP_3 turnover resulting in accumulation of IP_2 and IP_1. Thus, the study of radiolabeled inositol in CHO cells that have expressed human NK_1 receptors can serve as a functional assay.

Human NK_1/CHO cells were prelabeled with $[^3H]$-2-myo-inositol and incubated with substance P in the presence and absence of L-754,030. Cells were sonicated and inositol-1-phosphate (IP_1) was eluted and radioactivity was counted. Substance P produced a dose-related accumulation of IP_1. L-754,030 inhibited the substance P-induced accumulation of inositol-1-phosphate.

Effects of L-754,030 and L-758,298 on NK_1-receptor mediated smooth muscle contractions

In vitro preparations of guinea pig ileum longitudinal muscle/ mesenteric plexus (LM/MP) were placed in organ baths containing Krebs solution that was aerated with 95% O_2/5% CO_2 and maintained at 37°C. A resting tension of 0.5 g was applied to each isolated ileum strip. Contractile responses were produced by the selective NK_1-receptor agonist substance P-O-methyl ester (SPOMe). Concentration-effect curves were obtained with SPOMe. Concentration-effect curves were also obtained with SPOMe in the presence of either L-758,298 (1 and 100 nM) or L-754,030 (0.1, 0.3 and 1 nm); responses were expressed as a percentage of the maximum response to SPOMe alone.

The 1 nM concentration of L-758,298 produced nearly complete inhibition of SPOMe-induced contractile responses. The 1 nM concentration of L-754,030 produced an approximate 30% reduction in the maximum SPOMe-induced contractile response; the 0.1 and 0.3 nM concentrations had no effect. Thus, L-758,298 and L-754,030 are NK_1 receptor antagonists at nM concentrations.

Effects of L-754,030 and L-758,298 on NK_2-receptor mediated smooth muscle contractions

In vitro preparations of guinea pig tracheal rings were placed in organ baths containing Krebs solution that was aerated with 95% O_2/5% CO_2 and maintained at 37° C. A resting tension of 1 g was applied to each isolated tracheal ring. Contractile responses were produced by the selective NK_2-receptor agonist Nle^{10}-neurotensin A (4-10) [Nle^{10}-NKA(4-10)]. Concentration-effect curves were obtained with Nle^{10}-NKA(4-10). Concentration-effect curves were also obtained with Nle^{10}-NKA(4-10) in the presence of either L-758,298 (1 μM) or L-754,030 (1 μM); responses were expressed as a percentage of the maximum response to Nle^{10}-NKA(4-10) alone.

Neither L-758,298 nor L-754,030 had any effect on Nle^{10}-NKA(4-10) induced contractile responses at μM concentrations. Thus, L-758,298 and L-754,030 are not relatively potent antagonists at NK_2 receptors.

Effects of L-754,030 and L-758,298 on NK_3-receptor mediated depolarization of guinea pig superior cervical ganglia

Superior cervical ganglia were isolated from male guinea pigs, desheathed and placed in a grease-gap recording chamber. Ganglia were perfused with a physiological salt solution that was aerated with 95% O_2/5% CO_2 and maintained at 37° C. Depolarization of the ganglia was produced by the selective NK_3-receptor agonist senktide. Concentration-effects curves were obtained with senktide. Concentration-effect curves were also obtained with senktide in the presence of either L-758,298 (1 μM) or L-754,030 (1 μM); responses were expressed as a percentage of the maximum response to senktide alone.

Neither L-758,298 nor L-754,030 had any effect on senktide-induced depolarization responses at μM concentrations. Thus, L-758,298 and L-754,030 are not relatively potent antagonists at NK_3 receptors.

Mechanism of Action:

Fosaprepitant (L-758,298) is a pro-drug of aprepitant (MK-0869/L-754,030) and is rapidly converted to aprepitant following i.v. administration. Aprepitant is a Neurokinin$_1$ (NK_1; Substance P) receptor antagonist. Substance P-containing vagal afferent fibers innervate the brainstem nucleus, tactus solitarius, which is the region associated with emesis. Exogenous Substance P produces excitatory effects in cells of this region, most likely by acting on the NK_1 receptors. Aprepitant has been shown to bind selectively to NK_1 receptors from the rat, guinea pig, ferret and human. It has also been shown to inhibit the Substance P-induced inositol tris-phosphate (IP_3) synthesis in Chinese hamster ovary cells expressing NK_1 receptors. Thus, the anti-emetic effect of fosaprepitant/aprepitant may be due to the antagonism of NK1 receptors at the central level.

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In Vivo Studies:

Inhibition of cisplatin-induced emesis by L-758,298 and L-754,030 in ferrets

In one study, male ferrets were fed approximately 100 g of commercially available cat food, not less than 20 min before drug treatment. Under halothane anesthesia, either L-758,298 (0, 0.1, 0.3, 1 and 3 mg/kg) or L-754,030 (0, 0.1, 0.3, 1 and 3 mg/kg) were intravenously administered via the left jugular vein; 10 min later, cisplatin (10 mg/kg) was intravenously administered via the left jugular vein.

In a second study, ferrets were fed 10 min after oral administration of either L-758,298 (0, 0.3, 1 and 3 mg/kg) or L-754,030 (0, 0.3, 1 and 3 mg/kg). Fifty min later, cisplatin (10 mg/kg) was intravenously administered via the left jugular vein under halothane anesthesia.

Animals were observed continuously during recovery from anesthesia and for 4 hrs following cisplatin administration. As shown in the following table, intravenously administered L-758,298 and L-754,030 dose-dependently increased cisplatin-induced latency to retching and vomiting and reduced numbers of retches and vomits.

Inhibition of cisplatin-induced emesis (10 mg/kg, i.v.) by intravenously administered L-758,298 and L-754,030 in ferrets

Parameter	L-758,298 (mg/kg, i.v.)					L-754,030 (mg/kg, i.v.)				
	0	0.1	0.3	1	3	0	0.1	0.3	1	3
Latency (min)	75	70	80	210	240	85	75	90	240	240
No. Retches	170	120	40	5	0	105	95	5	0	0
No. Vomits	20	16	4	1	0	13	13	2	0	0

As shown in the following table, orally administered L-758,298 and L-754,030 dose-dependently increased cisplatin-induced latency to retching and vomiting and reduced numbers of retches and vomits.

Inhibition of cisplatin-induced emesis (10 mg/kg, i.v.) by orally administered L-758,298 and L-754,030 in ferrets

Parameter	L-758,298 (mg/kg, p.o.)				L-754,030 (mg/kg, p.o.)			
	0	0.3	1	3	0	0.3	1	3
Latency (min)	90	115	205	210	70	95	70	160
No. Retches	95	20	0	0	155	55	20	5
No. Vomits	12	6	1	0	22	7	4	1

Inhibition of cisplatin-induced emesis by combined treatment with L-754,030 and ondansetron (5-HT_3 receptor antagonist)

Male ferrets were fed approximately 100 g of commercially available cat food, not less than 20 min before drug treatment. Under halothane anesthesia, 4 groups were treated with water (1 ml/kg, i.v.) + PEG_{300} (1 mg/kg, i.v.), ondansetron (0.1 mg/kg, i.v.) + PEG_{300} (1 mg/kg, i.v.), water (1 ml/kg, i.v.) + L-754,030 (0.1 mg/kg, i.v.), and ondansetron (0.1 mg/kg, i.v.) + L-754,030 (0.1 mg/kg, i.v.), respectively. Three min later, cisplatin (10 mg/kg) was intravenously administered via the left jugular vein.

Animals were observed continuously during recovery from anesthesia and for 4 hrs following cisplatin administration. As shown in the following table, the anti-emetic effects of ondansetron and L-754,030 were additive.

Inhibition of cisplatin-induced emesis (10 mg/kg, i.v.) by intravenously administered L-754,030 plus ondansetron in ferrets

Parameter	Treatment			
	Vehicle	Ondansetron (0.1 mg/kg, i.v.)	L-754,030 (0.1 mg/kg, i.v.)	Ondansetron + L-754,030
Latency (min)	75	135	70	190
No. Retches	145	115	85	20
No. Vomits	19	18	15	2

Studies to Characterize the Duration of Anti-Emetic Activity of Aprepitant as a Functional Measure of its Receptor Kinetics *In Vivo*

The duration of action of aprepitant as an index of its *in vivo* receptor kinetics was investigated in the ferret cisplatin-induced model of emesis by varying its pre-treatment time before a standardized i.v. cisplatin challenge. Male ferrets were pre-treated with vehicle or aprepitant (3 mg/kg, p.o.) at 1, 12, 24, 48 and 72 hours before administration of cisplatin (10 mg/kg, i.v.). Retching and vomiting were recorded for 8 hours after cisplatin administration. Control animals were administered the vehicle (1 ml/kg 10% v/v ethanol, 30% v/v water, 60% v/v propylene glycol) at either 1 or 48 hr before cisplatin administration.

Aprepitant, administered at 1 hr, 12 hr or 24 hr before cisplatin administration, provided a similar degree of anti-emetic effect at all time points. In the 48-hour pre-treatment period, there was a lower anti-emetic protection, while in the 72-hour pre-treatment group, no protection was observed, and the emetic response not significantly different from the vehicle control. The results indicate that aprepitant maintains its anti-emetic activity for 24 hours after dosing, and repeated dosing of aprepitant is required to maintain CNS NK_1 receptor occupancy and sustain optimal anti-emetic efficacy. The anti-emetic effects of aprepitant (3 mg/kg) in ferrets at different pre-treatment times are summarized in the Table below.

Table 1 Effect of Pretreatment Time with Aprepitant (3 mg/kg P.O.) on the Inhibition of Cisplatin-Induced Retching and Vomiting in Male Ferrets

Pretreatment	Protected / T	Number of Retches	Number of Vomits
Vehicle	0 / 8	137.6 ± 18.8	13.5 ± 1.5
1 hr	1 / 3	3.7 ± 2.3	2.0 ± 1.5
12 hr	3 / 5	3.2 ± 2.2	2.4 ± 1.7
24 hr	2 / 3	1.0 ± 1.0	0.3 ± 0.3
48 hr	0 / 3	38.7 ± 14.8	4.7 ± 2.2
72 hr	0 / 3	108.0 ± 28.6	11.0 ± 1.5

Inhibition of cisplatin-induced emesis by combined treatment with L-754,030 and dexamethasone

Previous studies have shown that anti-emetic effects of ondansetron are potentiated by coadministration of dexamethasone (Hawthorn and Cunningham, 1990; Smith *et al*, 1991). Thus, it was of interest to investigate interactions of L-754,030 and dexamethasone.

Male ferrets were fed approximately 100 g of commercially available cat food, not less than 20 min before drug treatment. Under halothane anesthesia, 4 groups were treated with saline (1 ml/kg, i.v.) + PEG_{300} (1 mg/kg, i.v.), dexamethasone (20 mg/kg, i.v.) + PEG_{300} (1 mg/kg, i.v.), saline (1 ml/kg, i.v.) + L-754,030 (0.1 mg/kg, i.v.), and dexamethasone (20 mg/kg, i.v.) + L-754,030 (0.1 mg/kg, i.v.), respectively. Three min later, cisplatin (10 mg/kg) was intravenously administered via the left jugular vein.

Animals were observed continuously during recovery from anesthesia and for 4 hrs following cisplatin administration. As shown in the following table, the suppressant effect of L-754,030 on numbers of retches and vomits was potentiated by dexamethasone.

Inhibition of cisplatin-induced emesis (10 mg/kg, i.v.) by intravenously administered L-754,030 plus dexamethasone in ferrets

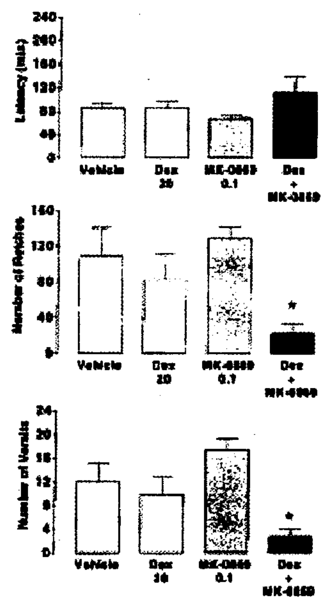
Parameter	Treatment			
	Vehicle	Dexamethasone (20 mg/kg, i.v.)	L-754,030 (0.1 mg/kg, i.v.)	Dexamethasone + L-754,030 -
Latency (min)	70	140	80	160
No. Retches	150	40	55	15
No. Vomits	19	6	8	2

Inhibitory Effects of Combined Treatment with MK-0869 and the Steroid Dexamethasone on Cisplatin-Induced Emesis in Ferrets.

Methods: To find whether the anti-emetic effect of MK-0869 is enhanced by co-administration of dexamethasone, the effects of combined treatment of these two agents were examined on Cisplatin induced vomiting in ferrets. Three groups of anesthetized animals received the vehicle, dexamethasone alone (20 mg/kg i.v.), MK-0869 alone (0.1 mg/kg i.v.) or dexamethasone (20 mg/kg) plus MK-0869 (0.1 mg/kg), 3 minutes before Cisplatin challenge (10 mg/kg i.v.). The animals were observed continuously for 4 hours after Cisplatin administration and the number of retches and vomits were recorded.

Results: Neither MK-0869 nor dexamethasone, when administered alone, had any effect on Cisplatin-induced retching and vomiting in ferrets. However, co-administration of dexamethasone with MK-0869 caused significant reductions in the numbers of retches and vomits after Cisplatin administration (P<0.05). Thus, single suboptimal doses of either agent alone were not effective on Cisplatin-induced vomiting in ferrets, while the combination was effective. The effects of dexamethasone and MK-0869, either alone or in combination, on Cisplatin-induced vomiting in ferrets is shown in the sponsor's Figure below.

Effect of Co-Administration of MK-0869 (0.1 mg/kg IV) with Dexamethasone (20 mg/kg IV) on the Retching and Vomiting Response to Cisplatin (10 mg/kg IV) in Ferrets



Values are means ± SEM (n = 6 per group); *p <0.05 compared with vehicle-treated ferrets (Dunnett's t-test).

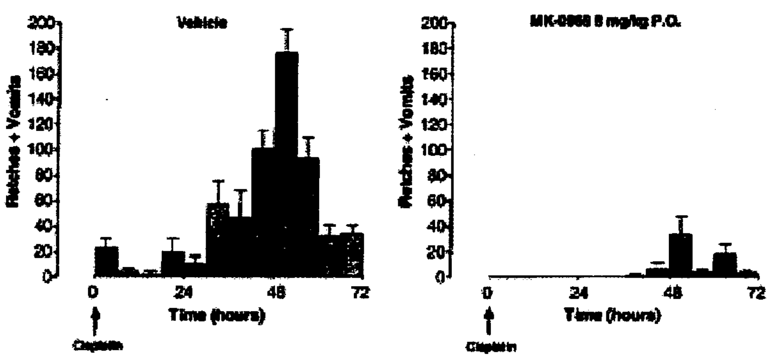
Inhibition of Cisplatin-Induced Delayed Emesis in Ferrets by MK-0869.

Methods: The study was conducted to investigate the ability of MK-0869 to inhibit the retching and vomiting in a model of delayed chemotherapy-induced emesis in the ferret. In this delayed emesis assay, ferrets were observed for 72 hours after cisplatin treatment (5 mg/kg i.p) and a biphasic response with peaks in the retching and vomiting response occurring between 2 to 16 hours and 30 to 60 hours was detected. In three separate studies, male ferrets were dosed orally with single daily oral doses of MK-0869 or the vehicle control. In study 1, a single oral dose of MK-0869 (2 ml/kg) or the vehicle was administered before cisplatin treatment. In study 2, daily single doses of MK-0869 (4 and

16 mg/kg) were administered once 2 hours before cisplatin treatment. In one group, the potential usefulness of MK-0869 as a rescue therapy was explored by delaying the therapy until 24 hours after cisplatin. In this group, MK-0869 (4 mg/kg) was administered 24 and 48 hours after cisplatin or as daily doses of 1 and 2 mg/kg, starting 2 hours before cisplatin treatment.

Results: MK-0869, at oral doses of 2 and 4 mg/kg, caused complete prevention of retching and vomiting for the entire 72-hour observation period after cisplatin treatment. At the 1 mg/kg dose, there was significant reduction of retching and vomiting as compared with the vehicle control group. At oral doses of 4, 8 and 16 mg/kg, given as a single dose, 2 hours before cisplatin, it caused a dose-dependent reduction of retching and vomiting on each of the 3 days. MK-0869 (4 mg/kg) when administered 24 and 48 hours after cisplatin treatment, almost completely prevented the delayed phase of emesis. Thus, MK-0869 was effective in preventing both the acute and delayed emetic response to cisplatin in ferrets. The profile of the anti-emetic actions after a single 8 mg/kg dose of MK-0869 during the 72-hour observation period are shown in the sponsor's Figure below.

Profile of the Anti-Emetic Actions of MK-0869 After a Single Dose at 8 mg/kg P.O. In Ferrets During a 72 h Observation Period after Cisplatin (5 mg/kg IP)



Bars represent the mean numbers of retches + vomits ± SEM in 6 hour time intervals (n = 4).
(TP) Notebook/Page: 13099/291 and 292; 90937/3
Study Period: Oct - Nov 1996

Inhibition of apomorphine-induced and morphine-induced emesis by orally administered L-754,030 and L-758,298 in ferrets

Ondansetron and granisetron (5-HT_3 receptor antagonists) are effective anti-emetics for cytotoxic chemotherapeutic agents, but are ineffective against morphine-induced and apomorphine-induced emesis (Andrews and Bhandari, 1993). However, the NK_1 receptor antagonist CP 99,994 has been shown to be an effective anti-emetic against morphine and apomorphine (Bountra et al., 1993; Tattersall et al., 1994). Thus, it was of interest to study the effects of L-754,030 and L-758,298 on morphine-induced and apomorphine-induced emesis.

Male ferrets were orally administered either 0.5% Methocel (1 ml/kg), L-754,030 (3 mg/kg) or L-758,298 (3 mg/kg). Sixty min later, animals were given either apomorphine (0.25 mg/kg, s.c.) or morphine (0.5 mg/kg). Animals were observed for retching and vomiting for 30 min.

As shown in the following table, both L-754,030 and L-758,298 inhibited apomorphine-induced emesis.

Inhibition of apomorphine-induced (0.25 mg/kg, s.c.) emesis by orally administered L-754,030 and L-758,298 in ferrets

Parameter	Treatment		
	Vehicle	L-754,030 (3 mg/kg, p.o.)	L-758,298 (3 mg/kg, p.o.)
Latency (min)	8.5	18	26
No. Retches	29	3	2
No. Vomits	3	1	0.3

As shown in the following table, both L-754,030 and L-758,298 inhibited morphine-induced emesis.

Inhibition of morphine-induced (0.5 mg/kg, s.c.) emesis by orally administered L-754,030 and L-758,298 in ferrets

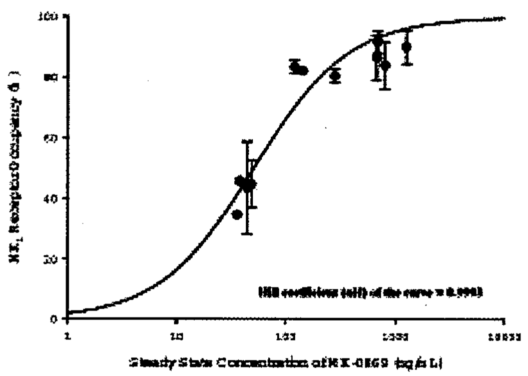
Parameter	Treatment		
	Vehicle	L-754,030 (3 mg/kg, p.o.)	L-758,298 (3 mg/kg, p.o.)
Latency (min)	6.5	19	19.5
No. Retches	47	7	16
No. Vomits	6	0.5	2

Determination of Central NK1 Receptor Occupancy by MK-0869 Using Positron Emission Tomography (PET) in Rhesus Monkeys.

Methods: NK_1 receptor occupancy of intravenous MK-0869 was investigated in monkeys using the PET radiotracer [^{18}F]L-829165 in vivo. The animals received L-758298 (a phosphoryl prodrug form of MK-0869) (a) by bolus plus constant i.v. infusion to achieve steady state plasma levels and (b) a single bolus i.v. dosing. PET studies were carried out in three monkeys at three different plasma steady state levels in each monkey and another monkey at one plasma steady state level of MK-0869. For the bolus protocol, plasma samples were collected at 0, 10, 20 and 30 minutes and at 30 minutes intervals for 240 minutes after the administration of MK-0869. For the bolus-plus-infusion protocols, plasma samples were collected at 0, 15 and 30 minutes and at 30 minutes interval until the end of the PET study (330 minutes from the beginning of the protocol). The peak NK_1 receptor occupancy after bolus dosing of 3 or 5 mg/kg of L-758298 was determined in four monkeys. Measurements were made at three time periods after dosing (10, 30 and 60 minutes). PET scans were performed on an ECAT EXACT HR+ scanner that allows the whole brain to be imaged. Drug receptor occupancy (%) was calculated as:
Percent occupancy = 100 x ($1-BP_{blockade}/BP_{baseline}$), where $BP_{baseline}$ and $BP_{blockade}$ refers to binding potentials before and after drug treatment, respectively.

Results: The steady state plasma concentrations achieved in four monkeys ranged from 36-1260 ng/ml while the corresponding NK_1 receptor occupancy ranged from 35% to 92%. When the PET occupancy results from the four monkeys were combined, a relationship between PET occupancy and the steady state plasma concentrations was observed. A 50% occupancy of the central NK_1 receptors was observed at 51 ng/ml and a 90% occupancy was observed at 470 ng/ml of the plasma drug concentration. A point estimate of the central receptor occupancy by MK-0869 after bolus i.v. administration of L-758298 could not be obtained because the plasma drug concentration and the receptor occupancy was constantly changing over the 180 min PET scan period. The average receptor occupancy after the 3 mg/kg i.v. bolus dose was about 90% in all four animals. In all bolus dose studies, the average plasma MK-0869 levels during the PET scan were higher than that established to achieve 90% receptor occupancy in the steady state experiments. The studies show that after i.v. administration of L-758298 (phosphorylated prodrug) to rhesus monkeys, a good relationship existed between the plasma MK-0869 concentrations and central NK_1 receptor occupancy. The relationship between the steady state plasma MK-0869 concentrations and central NK_1 receptor occupancy is shown in the sponsor's Figure below.

Relationship between Steady State Plasma Concentrations of MK-0869 and PET NK_1 Receptor Occupancy in Rhesus Monkeys



Note:
1) Data represent mean ± SD for the combined left and right striatal regions averaged over the 120 - 300 minute interval

2.6.2.3 Secondary pharmacodynamics

<u>Inhibition of centrally-medicated NK_1 agonist-induced foot tapping in gerbils</u>

Mongolian gerbils were anesthetized with isoflurane and intravenously administered either L-758,298 (0.1, 1 and 10 mg/kg) or L-754,030 (0.1, 1 and 10 mg/kg), followed by intracerebroventricular injection of the selective NK_1 agonist GR73632 (3 pmol).

b(4)

Immediately after recovery of the righting reflex, duration of GR73632-induced foot tapping was determined during a 5 min observation period. The dose of either L 758,298 or L-754,030 that reduced the duration of foot tapping by 50% of that observed in vehicle-treated animals (ID_{50}) was calculated by the software program GraFit.

The ID_{50}s were 0.32 and 0.95 mg/kg for L-754,030 and L-758,298, respectively.

<u>Inhibition of substance P-induced dermal inflammation in the guinea pig</u>

When dermal plasma extravasation was induced in the guinea pig by substance P (0.5 pmol/site), the ID_{50} and ID_{90} for orally administered L-754,030 were 0.052 and 0.37 mg/kg, respectively; the ID_{50} and ID_{90} for orally administered L-758,298 were 6 and

20 µg/kg, respectively. L-754,030 and L-758,298 (10 mg/kg, i.p.) inhibited substance P-induced (0.5 pmol/site) dermal plasma extravasation, but not histamine-induced (1 nmol/site) and PAF (10 pmol/site) dermal plasma extravasation.

Inhibition of Centrally-Mediated NK1 Agonist-Induced Foot Tapping in Gerbils by the Major Metabolites of MK-0869

The ability of major metabolites of MK-0869 to inhibit foot tapping in gerbils was determined at 2 hr or 24 hr prior to central NK_1 agonist challenge. Anesthetized male and female Mongolian gerbils were used in the study. The highly selective NK_1 receptor agonist GR73632 (3 pmol in 5 µl) was administered by intra cerebroventricular injection directly into the lateral ventricles. MK-0869 metabolites, L-755446, L-829617 and L-825678 were administered intravenously at 1, 3, 6 or 10 mg/kg, and the dose causing a 50% reduction in the duration of foot tapping was determined (ID_{50}). **b(4)**

Pretreatment with MK-0869 up to 24 hours caused a potent inhibition of GR73632-induced foot tapping in gerbils with an ID_{50} of ≤ 0.33 mg/kg. None of the metabolites tested had activity comparable to MK-0869. L-829617 was inactive at all pretreatment times. L-825678 had an ID_{50} value of 1.03 mg/kg when given immediately before GR73632, and the inhibition fell to only 12.4% and 2% when the 3 mg/kg dose was administered 4 or 24 hours pretreatment, respectively. The ID_{50} of L-755446 was approximately 1 mg/kg when administered immediately before GR73632 and 3 mg/kg after a 24 hr pretreatment.

Thus, MK-0869 was considerably more potent than the major metabolites in the gerbil foot tapping assay. The effect of MK-0869 and its metabolites on GR73632-induced foot tapping in gerbils is summarized in the Table below.

Inhibition of GR73632-induced Foot Tapping in Gerbils by MK-0869 and its major metabolites

Compound	Immediate	4h	24h
Parent MK-0869	0.32	0.04	0.33
L-829617	3% @ 10 mg/kg	3.2% @ 10 mg/kg	4.9% @ 10 mg/kg
L-825678	1.03	12.4% @ 3 mg/kg	2% @ 3 mg/kg
L-755446	53% @ 1 mg/kg	ND	49.5% @ 3 mg/kg

ND: not determined

Values are the mean % inhibition or ID_{50} values (mg/kg IV) for the prevention of foot-tapping induced in gerbils by GR73632. Test compounds were administered IV immediately, 4h or 24h before GR73632.
(n = 3-5 animals at each dose)

Inhibition of resiniferatoxin-induced systemic vascular leak in the guinea pig

When esophageal plasma extravasation was induced in the guinea pig by resiniferatoxin (7 nmol/kg, i.v.), the ID_{50} for orally administered L-754,030 and L-758,298 (given 1 h before resiniferatoxin) was 12 and 16 μg/kg; the ID_{50} for intravenously administered L-754,030 and L-758,298 was 1.7 and 5.6 μg/kg respectively. When orally administered L-754,030 and L-758,298 was given 24 h before resiniferatoxin, the ID_{50} was 0.5 and 0.9 mg/kg, respectively.

Inhibition of substance P-induced increase in hindlimb flow in the rhesus monkey

Intra-arterial infusion of substance P in monkeys produced dose-dependent increases in skin hindlimb blood flow (ED_{50} of approximately 2-5 pmol/min) which was maximal at 30 pmol/min. Substance P also produced decreases in vascular resistance (quotient of mean arterial pressure over skin blood flow) of approximately -25% (% of difference from control). Intravenously administered L-754,030 (1 mg/kg) and L-758,298 (1 mg/kg) shifted the substance P dose-response curves for skin hindlimb blood flow to the right; ED_{50}s for substance P were approximately 200 and >1000 pmol/min, respectively. Intravenously administered L-754,030 and L-758,298 had similar effects on substance P-induced decreases in skin vascular resistance.

Plasma extravasation in the dura mater of the rat

When plasma extravasation was induced in the dura mater by electrical stimulation of the trigeminal ganglion (5 Hz, 5 msec, 1.2 mA, 5 min) in the rat, the ID_{50} for intravenously administered L-758,298 and L-254,030 was 106 and 18 μg/kg, respectively. Thus, L-758,298 and L-254,030 might be effective against migraine, if substance P-induced neurogenic inflammation in the dura mater plays a major role in the etiology of migraine.

Inhibition of a facilitated nociceptive spinal flexor reflex in rabbits

Since substance P is a purported neurotransmitter in peripheral nociceptive pathways, it was of interest to assess the effects of L-754,030 on a facilitated nociceptive spinal reflex in rabbits. A decerebrate rabbit preparation was made; the use of this model prevents any CNS-mediated influences on spinal reflexes. Single or multiunit recordings of motor neuron activity were measured in the femoris/semitendinous muscle. Motor neuron activity was facilitated by a conditioning stimulus applied to the receptive field of the motor unit in the area of skin innervated by the sural nerve. Intravenously administered L-754,030 inhibited (ID_{50} of 1.0 mg/kg) the facilitation of the nociceptive spinal flexor reflex.

Effects of L-758,298 and L-754,030 on calcium-entry in smooth muscle

In vitro preparations of guinea pig ileum longitudinal muscle/mesenteric plexus (LM/MP) were placed in organ baths containing Krebs solution that was aerated with 95% O_2/5% CO_2 and maintained at 37° C. A resting tension of 1 g was applied to each isolated ileum strip. Following 60 min of equilibration in the organ bath, ileum strips were placed in modified Krebs solution for 20 min; modified Krebs solution contained no calcium, a doubled magnesium concentration to maintain tissue viability, and a high KCl concentration to depolarize the ileum strips and open voltage-operated calcium channels. A concentration-effect curve was obtained for Ca-entry by adding $CaCl_2$ directly to the bath (The sponsor did not describe the methodology for measuring Ca-entry). A concentration-effect curve was also obtained for Ca-entry in the presence of either L-758,298 (1µM) or L-754,030 (1, 3 and 10 µM). The tissues were then placed back into normal Krebs solution and equilibrated for 60 min. Treatment with modified Krebs solution and $CaCl_2$ was repeated. The response to each concentration of calcium was expressed as percentage of the maximum of the control curve.

L-758, 298 had no effect on calcium entry. The highest concentration of L-754, 030 (10 µM) had a weak effect on calcium entry (K_a of 5.5 µM).

Assessment of 5-HT_{1D}-receptor-mediated effects:

Since selective 5-HT_{1D} receptor agonists have been shown to reduce dural plasma protein extravasation induced by electrical stimulation of the trigeminal ganglia in anesthetized rats, and since NK_1-receptor antagonists have been shown to inhibit dural extravasation in this animal model, the present study assessed whether either L-758, 298 or L-754, 030 have 5-HT_{1D} receptor agonist vasoconstrictor properties.

In vitro preparations of rabbit saphenous vein ring segments were placed in organ baths containing Krebs solution that was aerated with 95% O_2/5% CO_2 and maintained at 37^0C. A resting tension of 2 g was applied to each isolated ring segment. The contractile response to 5-HT (1 µM) was determined. Cumulative concentration-effect curves were determined for sumatriptan (selective 5-HT_{1D} receptor agonist), L-758, 298 and L-754, 030; agonist potency (EC_{50}) and relative efficacy [response compared to that produced by 5-HT (1 µM)] were determined.

Sumatriptan had an EC_{50} of 0.6 µM and produced a maximum contraction of the ring segments that was similar to that produced by 5-HT. In contrast, L-758, 298 and L-754, 030 were devoid of any effect on the ring segments at concentrations up to 30 µM.

Inhibition of Centrally-Mediated NK_1 Agonist-Induced Foot-Tapping in Gerbils by the Major Metabolites of MK-0869.

Methods: Central infusion of NK_1 receptor agonists in gerbils elicits a vigorous, repetitive hind-foot tapping response. Foot tapping is blocked by administration of brain-penetrant NK_1 receptor antagonists. The highly selective NK_1 receptor agonist, GR73632 was administered via jugular vein after i.v administration of the test compounds (L-754030, L-755446, L-825678 and L-829617) at 1, 3, 6 or 10 mg/kg doses.

Results: None of the metabolites tested had inhibitory effects, comparable to MK-0869 (ID_{50}, ≤0.33 mg/kg) on GR73632-induced foot tapping in gerbils. L-829617 had no effect at any treatment time. L-825678 had an ID_{50} of 1.03 mg/kg when given immediately before GR73632, but the inhibition was much lower when administered at 3 and 24 hours prior to GR73632 administration. L-755446 was also active when administered immediately before the NK_1 agonist with an ID_{50} of 1 mg/kg.

Evaluation of MK-0869 and Metabolites as Potential Inhibitors of Human Liver Monoamine Oxidase A and B Activities.

Methods: Monoamine oxidase (MAO) activities were measured using pooled human liver S9 fractions as a source of MAO-A and MAO-B activities. Five-hydroxytryptamine (5-HT) and β-phenylethylamine (PEA) were used as selective substrates for MAO-A and MAO-B, respectively. Incubations were conducted in the presence or absence of potential MAO inhibitors in the presence of the enzyme prior to addition of the substrate. Compounds tested as potential inhibitors were MK-0869 (L-754030) and its metabolites, L-755446, L-809861, L-809771, L-825678, L-829615 and L-829617. The 5-HT and PEA metabolites were measured by HPLC analysis.

Results: MK-0869 and its metabolites did not cause any significant inhibition of MAO-A or MAO-B activity at concentrations up to 100 µM. The positive controls caused a significant inhibition of the enzymes activities.

Pharmacology Summary:

Fosaprepitant (L-758,298) is a pro-drug of aprepitant (MK-0869/L-754,030) and is rapidly converted to aprepitant following i.v. administration to experimental animals. After i.v. dosing of fosaprepitant to rhesus monkeys, a good correlation between plasma active drug (aprepitant) concentration and central receptor occupancy was observed. Aprepitant binds selectively and with high affinity to NK_1 receptors from different species. It causes inhibition of binding of $[^{125}I]$substance P to cloned human, rat, dog and guinea pig NK_1 receptors, and to NK_1 receptors from dog, monkey, mouse, rabbit and ferret cerebral cortex with IC_{50} values in the nM range. The specificity of binding of aprepitant to NK_1receptors has been shown by its ability to compete with L-703,606 (a selective NK_1 receptor antagonist) for the binding sites at human NK_1receptors. It inhibits substance P-induced IP_3 formation in cloned Chinese hamster ovary (CHO) cells expressing human NK_1 receptors. Intravenously administered aprepitant (1.0 mg/kg) caused an inhibition of facilitation of the nociceptive spinal flexor reflex in a rabbit model. Oral or i.v. aprepitant caused dose-dependent increases in the latency of cisplatin-induced retching and vomiting and reduced the number of retches and vomits in ferrets. Aprepitant, at oral doses of 2 and 4 mg/kg, caused a complete inhibition of cisplatin-induced delayed retching and vomiting in ferrets. An additive anti-emetic effect was observed when the drug was administered with dexamethasone.

Pharmacology Conclusions:

Substance P may have important roles in emesis, as substance P-containing vagal afferent fibers innervate the brainstem nucleus tactus solitarius, a region of the CNS involved in emesis. Fosaprepitant is a precursor of aprepitant which is a selective and potent neurokinin$_1$ (NK_1) receptor antagonist, as shown by its affinities for cloned and native NK_1 receptors from different species. The antiemetic effect of aprepitant has been assessed, either alone or in combination, in different animal models. It has been found to be very effective in preventing both the initial and delayed phases of cisplatin-induced emesis in ferrets. It had additive effects on the anti-emetic effects of ondansetron or dexamethasone in this animal model. Fosaprepitant, when administered intravenously to different animals, is very rapidly converted to aprepitant. Thus, intravenous fosaprepitant may be effective in preventing chemotherapy-induced emesis in cancer patients.

2.6.2.4 Safety pharmacology

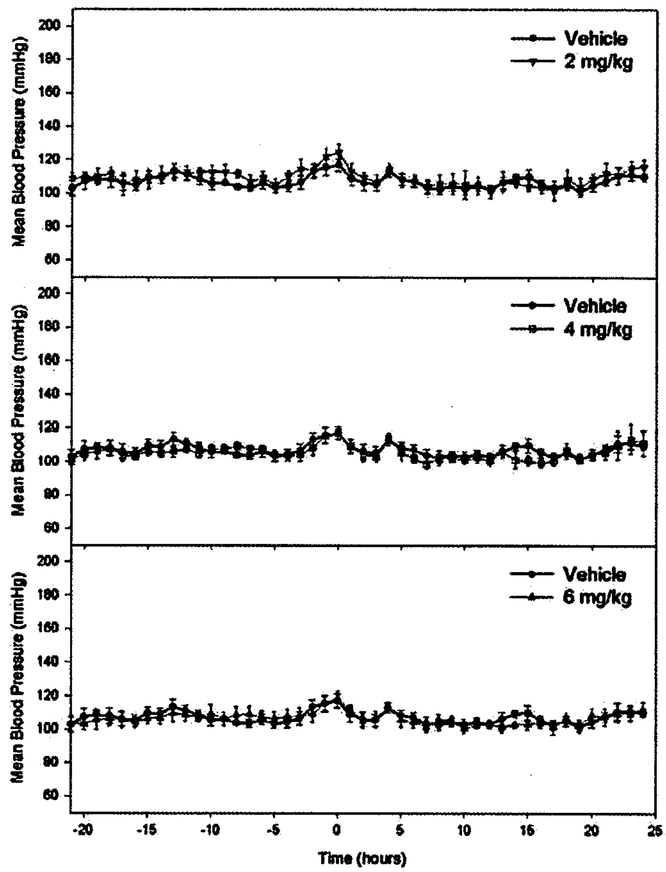
L-000758298: Cardiovascular Telemetry Assay in Dogs (Study # 04-5612

The cardiovascular effects of L-000758298 (Lot # L-000758298-003C016) was determined in conscious telemetered beagle dogs (1 female, 2 males) following intravenous administration of 2, 4 and 6 mg/kg doses. The study design was a 4 x 4 Latin square crossover. All animals received all doses with a ≥ week washout period. The dosing schedule for the 4 dogs is shown in the Table below.

		L-000758298 (mg/kg)			
	Animal Number	Dose Week 1	Dose Week 2	Dose Week 3	Dose Week 4
Female	4095481	6	4	2	Vehicle
Males	3941191	Vehicle	2	4	6
	3944379	4	Vehicle	6	2
	3936732	2	6	Vehicle	4

Lyophilized samples of L-000758298 were reconstituted in 0.9% sodium chloride for injection and administered at dosing volumes of 0.08, 0.16 and 0.24 ml/kg for 2, 4 and 6 mg/kg doses. Arterial blood pressure, heart rate, PR interval, QRS interval and QT interval were measured. QT interval was also reported as the rate corrected QT interval **using Fridericia's rate** correction formula. Data were collected for ≥ 18 hours prior to dosing and ≥ 24 hours post-dosing. Data were collected as 15 minute mean values. Blood samples (approximately 2 ml) were collected from all animals approximately 24 hours before each dosing and approximately 2 minutes post-dosing in Weeks 1, 2, 3 and 4 for determination of the plasma concentrations of L-000758298 and its active metabolite (L-000754030).

Treatment with L-000758298 had no effects on the systolic, diastolic and mean blood pressures of conscious dogs at i.v. doses up to 6 mg/kg. No treatment-related effects on the heart rate, QRS, QT or QTc intervals were observed at any doses examined. The effects of the vehicle and L-000758298 on the mean blood pressure and QT intervals are shown in the Figures below.



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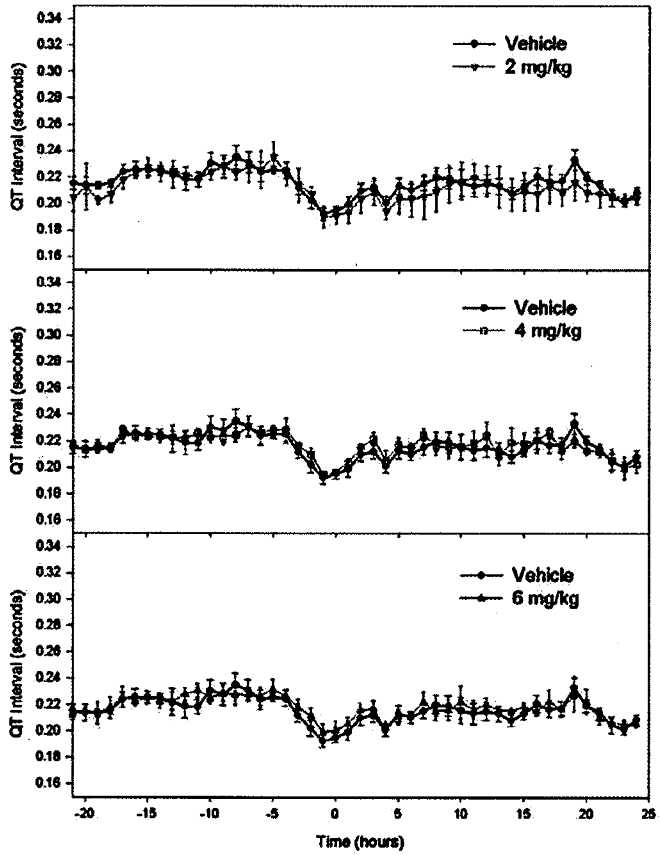


Plasma concentrations of L-000758298 (prodrug) and L-000754030 (active compound) increased with increasing dose. However, the plasma concentrations of individual animals were variable. The mean plasma concentrations of L-000758298 and L-000754030 in dogs receiving 2.0, 4.0 and 6.0 mg/kg doses are shown in the Tables below.

Table B-1. L-000758298: Cardiovascular Telemetry Assay in Dogs. TT #04-5612

Mean Plasma L-000758298 Concentrations (µg/mL)

Approximate Time Postdose (Hours)	L-000758298 (mg/kg)			
	Sexes Combined			
	Vehicle	2.0[a]	4.0	6.0
0[b]	0 ± ID[c]	0 ± ID[c]	0 ± ID[c]	0 ± ID[c]
0.0333	0 ± ID[c]	6.29 ± 3.86	7.85 ± 3.86	22.1 ± 14.3

Values are the mean ± SEM.
ID = Insufficient data available for calculation.
[a] N=3 due to one animal being removed due to misdosing.
[b] 0-hour samples were collected at ≥-18 hours before dosing.
[c] All individual values were below the LLQ (0.0100 µg/mL).

Table B-2. L-000758298: Cardiovascular Telemetry Assay in Dogs. TT #04-5612

Mean Plasma L-000754030 Concentrations (µg/mL)

Approximate Time Postdose (Hours)	L-000758298 (mg/kg)			
	Sexes Combined			
	Vehicle	2.0[a]	4.0	6.0
0[b]	0 ± ID[c]	0 ± ID[c]	0 ± ID[c]	0 ± ID[c]
0.0333	0 ± ID[c]	3.80 ± 2.33	5.35 ± 2.69	7.74 ± 4.51

Values are the mean ± SEM.
ID = Insufficient data available for calculation.
[a] N=3 due to one animal being removed due to misdosing.
[b] 0-hour samples were collected at ≥-18 hours before dosing.
[c] All individual values were below the LLQ (0.0100 µg/mL).

2a. Cardiovascular effects and autonomic interactions

Cardiovascular effects and autonomic interactions in anesthetized dogs

Dogs were anesthetized with sodium pentobarbital (30 to 35 mg/kg, i.v.), artificially respired and vagotomized. Changes in arterial pressure and heart rate were induced by central vagal stimulation (square wave pulses of 10 volts, 10 msec in duration, 30 per second for 10 sec), peripheral vagal stimulation (square wave pulses of 6 volts, 10 msec in duration, 30 per sec for 6 sec) and intravenous administration of methacholine (1 µg/kg), epinephrine (1.5 µg/kg), phenethylamine (100 µg/kg), McNeil-343-A (100 µg/kg), dimethylphenylpiperazinium iodide (25 µg/kg) and substance P (13.4 ng/kg). Effects of L-758,298 (1 mg/kg, i.v.) alone and in combination with the above were assessed.

L-758,298 alone had little or no effect on blood pressure, heart rate and ECG activity. The depressor response and tachycardia produced by substance P were reversed by L-758,298.

Cardiovascular responses produced by the electrical stimulation and the other agonists were not affected by L-758,298. Thus, L-758,298 inhibited the effects of substance P (NK_1 receptor antagonism), but had no anti-cholinergic, anti-adrenergic or ganglionic-blocking activity.

Inhibition of blood pressure and heart rate responses to substance P in anesthetized dogs

Dogs were anesthetized with sodium pentobarbital (30 to 35 mg/kg, i.v.) and artificially respired. Dose-response curves were generated for effects of substance P (0.1 to 1000 ng/kg, i.v.) on mean arterial pressure and heart rate after vehicle pretreatment (control). Dose-response curves were then generated for substance P after pretreatment with L-758,298 (0.01 and 0.1 mg/kg, i.v.). The Effective Dose of substance P that reduced mean arterial blood pressure by 25 mm (ED_{-25} mm Hg) was 0.33 ng/kg after vehicle pretreatment, and 19.46 and 715.43 ng/kg after pretreatment with 0.01 and 0.1 mg/kg of L-758,298, respectively. The Effective Dose of substance P that increased heart rate by 20 bpm (ED_{+20} bpm) was 0.28 ng/kg after vehicle pretreatment, and 13.91 and 170.67 ng/kg after pretreatment with 0.01 and 0.1 mg/kg of L-758,298, respectively. Thus, L-758,298 was a potent inhibitor of substance P.

2b. Renal function and electrolyte excretion

Renal function and electrolyte excretion in conscious dogs

Dogs were orally administered L-758,298 (5 mg/kg) and effects on glomerular filtration rate, urine flow, sodium excretion, potassium excretion, effective renal plasma flow, plasma sodium, plasma potassium, filtration fraction, hematocrit and urine pH and glucose were assessed for 180 min.

Baseline urine flow was 0.39 ml/min. After L-758,298 administration, urine flow gradually increased over 180 min and was 0.62 ml/min during the 150-180 min interval. Baseline sodium excretion was 57 µEq/min. After L-758,298 administration, sodium excretion gradually increased over 180 min and was 116 µEq/min during the 150-180 min interval. There were no other treatment-related effects.

2e. Behavioral and other CNS effects

Behavioral and other CNS effects in conscious mice

In a dose-ranging study, mice were orally administered L-758,298 (1, 30 and 100 mg/kg) and observed for clinical signs of toxicity for 30 min. There were no treatment-related effects.

Thus, mice were either orally administered vehicle (0.5% methylcellulose) or L-758,298 (100 mg/kg) and observed over 60 min for motor signs (activity on grid, posture, muscle tone, ataxia, grip strength, tremor, convulsions and psychomotor activation), autonomic signs (eye closure when handled, dyspnea, salivation, lacrimation, hypothermia, piloerection, abnormal skin color, exophthalmos, writhing and pupil size), reflexes (righting, startle, pinna and corneal) and death.

There were no treatment-related effects on any of these parameters.

In summary, receptor binding studies indicated that L-754,030 and L-758,298 have relatively potent and selective affinities for NK_1 (SP) receptors from rat, dog, guinea pig, ferret and human; while in a functional assay, L-754,030 inhibited SP-induced accumulation of inositol-1-phosphate. I.v. L-758,298 and L-754,030 dose-dependently inhibited δ-aminovaleryl-[L-Pro^3,N-$MeLeu^{10}$]-SP(7-11) (GE73632)-induced foot tapping in gerbils, and i.v. and oral L-758,298 dose-dependently inhibited cisplatin-induced emesis in ferrets. I.v. and oral L-754,030 and L-758,298 inhibited SP-induced dermal and esophageal plasma extravasation in the guinea pig, and inhibited SP-induced increases in skin hindlimb blood flow in monkeys. When plasma extravasation was induced in the dura mater of the rat by electrical stimulation, i.v. L-758,298 and L-754,030 were inhibitory.

On the other hand, L-758,298 had no effect on calcium-entry in guinea pig smooth muscle and had no effect on isolated rabbit saphenous vein ring segments. I.v. L-758,298 had no effect on cardiovascular responses in the anesthetized dog, but reversed the SP-induced depressor response and tachycardia. Oral L-758,298 increased urine flow and sodium excretion, but had no other renal effects in the anesthetized dog. I.v. L-758,298 had no effect on respiratory function and hemostasis in anesthetized dogs. Oral L-758,298 had no effect on gastrointestinal parameters in conscious dogs and mice. Finally, oral L-758,298 had no effect on motor signs, autonomic signs or reflexes in conscious mice.

Comparisons of L-754,030 and L-758,298 doses producing primary and secondary *in vivo* pharmacological effects are, in general, not possible because primary studies employed gerbils, ferrets, guinea pigs, monkeys and rats, while secondary studies employed dogs and mice.

L-000758298: Functional Observational Battery Assay (TT # 04-5615)

Sprague Dawley rats (59-60 days old; 5 animals/sex/group) were administered L-000758298 (Lot # L-000758298-010A001) at a single intravenous dose of 2.5, 5.0 or 7.5 mg/kg. The control group was administered the vehicle (0.9% sodium chloride). At approximately 15 minutes after dosing, the animals were tested for a functional observational battery which includes: clinical signs (locomotor activity, posture, gait, presence/absence convulsions, presence/absence stereotypy, number of rears, fecal boluses and urine pools), click response, approach response, touch response, pupil response, palpebral response, pinna response, and surface and aerial righting reflexes. In addition, the forelimb grip strength, hindlimb grip strength, body temperature, foot splay and body weight were also measured.

In the Functional Observational Battery Assay, L-000758298 had no treatment-related neurobehavioral effects in male and female rats at single intravenous doses up to 7.5 mg/kg.

2c. Respiratory function and hemostasis

Respiratory function and hemostasis in anesthetized dogs

Anesthetized dogs (sodium pentobarbital, 30 to 35 mg/kg, i.v.) were intravenously administered L-758,298 (1 mg/kg) and effects on peak expiratory flow, intrapulmonary pressure, tidal volume, compliance, airway resistance, respiratory rate, minute volume, mean arterial pressure, heart rate, systolic blood pressure, diastolic blood pressure and end expiratory work were assessed for 60 min.

There were no treatment-related effects on any of these parameters.

2d. Gastrointestinal effects

Gastric acid secretion in conscious dogs with chronic gastric fistulas

Dogs were surgically prepared with chronic gastric fistulas. Basal gastric acid secretion was measured; total acid output over 90 min was 0.03 μEq. Orally administered L-758,298 (5 mg/kg) had no effect on total acid output.

Dogs were pretreated with either L-758,298 (5 mg/kg, p.o.) or vehicle (DMSO). One hr later, each dog was subcutaneously administered gastrin (64 μg/kg). Gastrin-stimulated total acid output over 90 min was 9.98 μEq after vehicle pretreatment. Orally administered L-758,298 (5 mg/kg) had no effect on gastrin-stimulated total acid output.

Gastrointestinal motility in conscious mice

Female mice were pretreated with either orally administered L-758,298 (5 mg/kg) or vehicle (0.5% methylcellulose). One hr later, mice were orally administered an acacia/charcoal suspension (0.3 ml). Twenty min later, mice were sacrificed by cervical dislocation and the entire gastrointestinal tract was removed. The distance that the charcoal meal traveled in the small intestine was determined. There was no effect of L-758,298 pretreatment on the distance that the charcoal meal traveled in the small intestine. Thus, L-758,298 had no effect on gastrointestinal motility.

In mice that were pretreated with morphine (either 6 mg/kg, p.o. or 12.5 mg/kg, s.c.), gastrointestinal motility was decreased. In mice that were pretreated with neostigmine (0.032 mg/kg, s.c.) or N-methylscopolamine (1 mg/kg, s.c.), gastrointestinal motility was increased.

Safety Pharmacology Summary:

Fosaprepitant (L-758,298) had no effects on the blood pressure, heart rate or ECG parameters of anesthetized dogs at an i.v. dose of 1.0 mg/kg. In conscious telemetered dogs, L-758,298, at i.v. doses up to 6 mg/kg, had no effects on blood pressures, heart rate or ECG parameters. In anesthetized dogs, substance P-induced depressor response and tachycardia were reversed by L-758, 298. L-758, 298 had no behavioral or central nervous system (CNS) effects in conscious mice at oral doses up to 100 mg/kg. It had no treatment-related effects on the respiratory function (peak expiratory flow, intrapulmonary pressure, tidal volume, airway resistance, respiratory rate and minute volume) or hemostasis (mean arterial pressure, heart rate, systolic and diastolic blood pressures) of anesthetized dogs. L-758, 298 had no effects on gastric acid output of conscious dogs or on gastrointestinal motility of conscious mice.

Safety Pharmacology Conclusions:

The safety pharmacology studies conducted with L-758, 298 in different animal models do not indicate any potential safety concerns when administered at the therapeutically pharmacological doses.

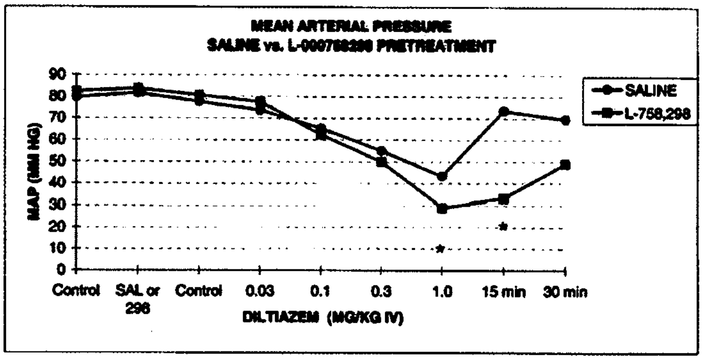
Other Studies:

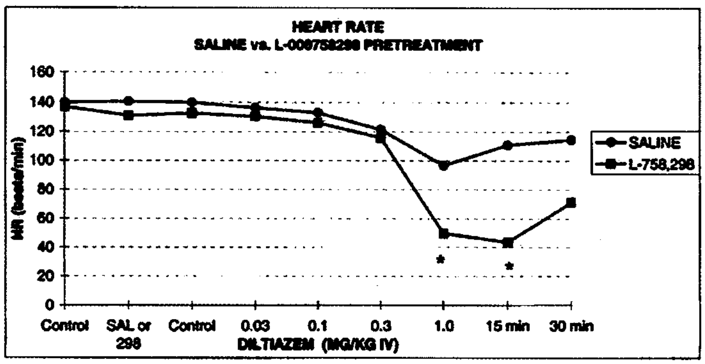
Effect of the Neurokinin (NK-1) Receptor Antagonist, L-000758298, 3.0 mg/kg IV, in Combination with the Calcium Channel Blocker, Diltiazem, on Hemodynamics in Anesthetized Dogs (Report L-000758298-225-3)

Anesthetized female mongrel dogs (n=6) were used in the study to determine the potential interaction of L-000758298 (3.0 mg/kg i.v.) with the calcium channel blocker, diltiazem (0.03 to 1.0 mg/kg, i.v.). The drug was administered via a femoral vein and the blood pressure was recorded from the right femoral artery. The effects of L-000758298 on diltiazem induced changes in heart rate (HR), systolic left ventricular pressure (LVP), left ventricular dP/dt max, left ventricular dP/dt min ECG (Lead II) PR interval were also examined.

L-000758298, at a single i.v. dose of 3.0 mg/kg, had no effects on the mean arterial pressure MAP), heart rate (HR), systolic left ventricular pressure (LVP), left ventricular dP/dt max, left ventricular dP/dt min or PR interval when compared with the vehicle. A transitory decrease in cardiac output (CO) and a transitory increase in the left ventricular end diastolic pressure (LVEDP) were observed after L-000758298 administration.
Diltiazem alone elicited the expected hemodynamic responses which include: decreases in MAP and heart rate, PR-interval prolongation (AV conduction delay) and modest cardiac depression (CO, LVP, dP/dt max and dP/dt min) with minor effects on LVEDP. No differences in the diltiazem curves, at doses up to 0.3 mg/kg, were observed in the presence or absence of L-000758198. However, at 1.0 mg/kg i.v. diltiazem, the effects were sustained longer in dogs pretreated with L000758298. ECG evidence of AV block with 1.0 mg/kg i.v. diltiazem was observed in all dogs, regardless of pretreatment. Blood pressure and heart rate responses to norepinephrine were not altered by L-000758298 (3.0 mg/kg i.v.). The effects of the vehicle and L-758298 on diltiazem-induced changes in mean arterial blood pressure, heart rate, PR interval, cardiac output, dP/dt max and dP/dt min are shown in the Figures below.

Effect of Vehicle (Saline, 2 mL IV) or L-000758298 (3.0 mg/kg IV) Alone and in Combination with Diltiazem on Mean Arterial Pressure and Heart Rate in Anesthetized Dogs (n=3/Group)





* $p<0.05$; saline vs. L-000758298

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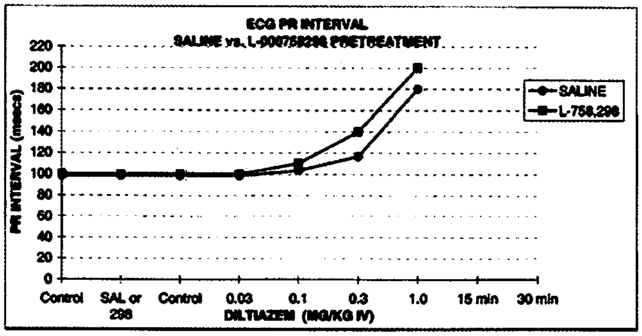
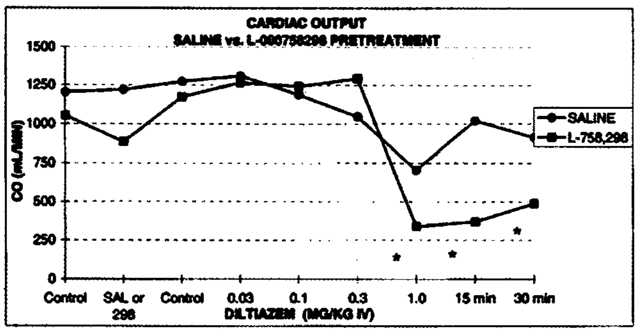
Effect of Vehicle (Saline, 2 mL IV) or L-000758298 (3.0 mg/kg IV) Alone and in Combination with Diltiazem on ECG PR Interval and Cardiac Output in Anesthetized Dogs (n=3/Group)



AV conduction delay observed in all dogs after 1.0 mg/kg IV diltiazem



* $p<0.05$; saline vs. L-000758298

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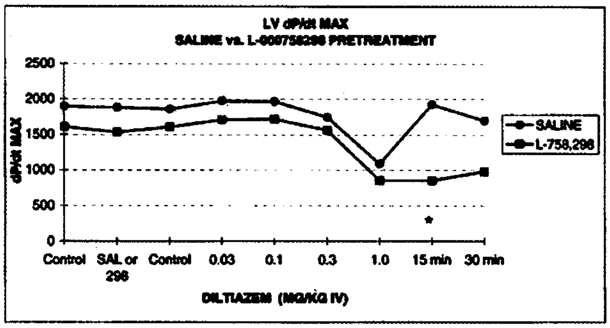
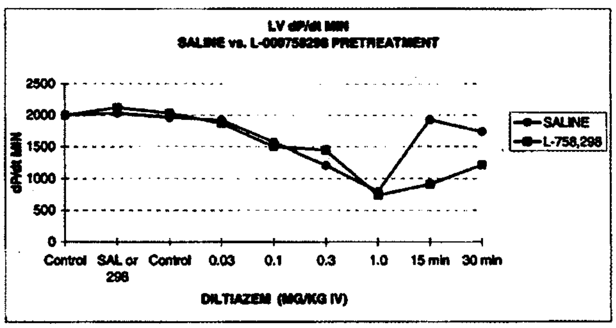
Effect of Vehicle (Saline, 2 mL IV) or L-000758298 (3.0 mg/kg IV) Alone and in Combination with Diltiazem on Left Ventricular dP/dt Max and dP/dt Min in Anesthetized Dogs (n=3/Group)





* $p<0.05$; saline vs. L-000758298

The mean plasma concentrations of L-000758298 and its active metabolite, L-000754030 were 410 and 3675 ng/ml, respectively, at 5 minutes following i.v. administration of a 3.0 mg/kg dose. This indicates that L-000758298 is rapidly converted to its active metabolite following i.v. administration in dogs. At the 1.0 mg/kg diltiazem dose, the plasma diltiazem concentrations were slightly higher (720 vs. 551 ng/ml) in animals pretreated with L-000758298, when compared to those pretreated with the vehicle.

Thus, there was no potentiation of the calcium channel blocking activity of diltiazem by i.v. L-000758298 (3.0 mg/kg). At the largest diltiazem dose (1.0 mg/kg, i.v.), the duration and magnitude of several diltiazem responses were slightly increased in the presence of L-000758298. This could be due to reduced plasma clearance of diltiazem by L-000758198.

MK-0869 Chemotherapy-Interaction Study:

Methods: As MK-0869 will be administered concurrently with cisplatin in cancer patients, the potential interactions of MK-0869 and cisplatin were studied in mice after co-administration of these two agents. Athymic NCr-nu male mice bearing a human small cell lung carcinoma xenograft (NCI-H82 cell line) were used in the study, and the effects of the NK_1 receptor antagonists on tumor growth and their potential to modify the anti-tumor activity and toxicity of cisplatin were studied. Two separate interaction studies were conducted. The first study included six groups of animals and received the following treatments:
group-1, no treatment; group-2, 3 mg/kg i.p. cisplatin; group-3, 0 mg/kg i.p. L-760735 (active NK_1 receptor antagonist), group-4, 10 mg/kg i.p. L-760735 plus 3 mg/kg i.p cisplatin; group-5, 10 mg/kg i.p L-781773 (NK_1 receptor inactive enantiomer of L-760735); group-6, 10 mg/kg i.p L-781773 plus 3 mg/kg i.p. cisplatin. Treatment was initiated on day 13 after tumor initiation and the vehicle or the agents were administered 1 hour before cisplatin on days 13, 17 and 21.
The second study included nine groups of animals and received the following treatments: group-1, no treatment; group-2, 3 mg/kg i.p. cisplatin; group-3, 6 mg/kg i.p. cisplatin; group-4, 10 mg/kg i.p. MK-0869 plus 6 mg/kg i.p. cisplatin; group-5, 10 mg/kg i.p. MK-0869 plus 3 mg/kg i.p. cisplatin; group-6, 10 mg/kg i.p. cisplatin; group-7, 10 mg/kg i.p. L-759274 (inactive enantiomer) plus 6 mg/kg i.p. cisplatin; group8, 10 mg/kg i.p. L-759274 plus 3 mg/kg i.p. cisplatin; group-9, 10 mg/kg i.p. L-759274. In this study, the treatment was initiated on day 17 after tumor implantation and the vehicle or the agents were administered 1 hr before cisplatin on days 17, 21 and 25.

Results: Treatment of the mice with cisplatin and the NK_1 receptor antagonists alone or in combinations had no adverse effect on the body weights or mortality. In the first study, 3 mg/kg i.p cisplatin showed moderate activity against the tumor. The combination of L-760735 and its inactive enantiomer, L-781773 with cisplatin showed no inhibitory effect on cisplatin. Cisplatin (3 mg/kg) alone caused 54% suppression of the tumor growth, and the combinations of cisplatin with L-760735 and L-781773 caused 46% and 39% suppressions of the tumor growth, respectively. In the second study, 6 mg/kg of i.p. cisplatin was effective against the tumor, while the 3 mg/kg dose was not effective. Thus, the NK_1 receptor antagonist, MK-0869 or its inactive enantiomer had no effect on the tumor growth by themselves or on the tumor inhibitory effects of 6 mg/kg cisplatin. Although, the intended clinical route is the oral route, the studies were conducted by administering the NK_1 receptor antagonists by the i.p. route.

2.6.2.5 Pharmacodynamic drug interactions

The preclinical studies conducted with L-758,298 do not indicate any potential for pharmacodynamic drug interactions.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

N/A

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary:

Pharmacokinetic/toxicokinetic studies were conducted with both fosaprepitant (L-758, 298; pro-drug) and the active drug (aprepitant; L-754, 030/MK-0869) following i.v. and oral administration to rats, mice, dogs and ferrets. Fosaprepitant (L-758,298) was rapidly converted to aprepitant in *in vitro* liver preparations from rat, dog and humans and by the S9 fractions from human lung, kidney and ileum, suggesting that the conversion of the pro-drug to active drug can take place in extrahepatic tissues. L-758,298 was rapidly (C_{max} was reached in 2 minutes) converted to its active metabolite, aprepitant following i.v. administration in rats and dogs. In humans, fosaprepitant administered intravenously, was rapidly converted to aprepitant within 30 minutes following the end of infusion. Following i.v. dosing of a 0.5 mg/kg dose to ferrets, the half-life, Cl and Vd_{ss} values were 10 hr, 1.5 ml/kg/min and 1.3 L/kg, respectively. The terminal half-life of intravenous aprepitant in rats was approximately 3 hours. In humans, the apparent terminal half-life of aprepitant ranged from approximately 9 to 13 hours. The clearance of MK-0869 was lower in ferrets as compared with rats and dogs. It had high binding affinities for plasma proteins from rats, dogs and humans (>98%). It penetrated the blood brain barrier in rats and ferrets following i.v. and oral administration, and was excreted in the milk of lactating rats following oral dosing. In human liver microsomes, CYP3A4 was the major CYP enzyme involved in the metabolism of MK-0869. Two other CYP enzymes, CYP1A2 and CYP2C19 were also involved in the metabolism of the compound. Fecal excretion was the major excretory pathway in rats, while in dogs fecal excretion was similar to urinary excretion following i.v. administration.

2.6.4.2 Methods of Analysis

The concentrations of the drug and its metabolites in the plasma, urine or feces were determined by a liquid chromatography tandem mass spectrometry (LC/MS/MS) method.

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2.6.4.3 Absorption

Rats

1. Pharmacokinetics of intravenously administered L-754,030 and L-758,298

Animals: Male Sprague-Dawley rats (body weight range of 300-400 g; ages were not provided by the sponsor).

Methods: L-758,298 was dissolved in a solution of lactose (50 mg/ml), potassium chloride (1.38 mg/ml), citric acid monohydrate (0.85 mg/ml) and sodium chloride (4 mg/ml) (pH 7.0); dosing volumes were 1, 8 and 25 mg/ml of L-758,298, respectively. L-754,030 was prepared in a solution of ethanol:propylene glycol:water (15:60:25,v/v/v) or a solution of PEG_{400}:water: ethanol (60:20:20, v/v/v); dosing volume was 0.4, 2 and 2 mg/ml of L-258,030, respectively.

Rats (4 per dose) were intravenously administered either L-758,298 (1, 8 and 25 mg/kg) or L-754,030 (0.2, 2 and 5 mg/kg) via the jugular vein. Blood samples were withdrawn from the femoral vein at 0.042, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 30, 48, and 72 hr after dosing. Plasma concentrations of L-754,030 and L-758,298 were determined by a LC/MS/MS assay.

Results: As shown in the following table, there were dose-related increases in $AUC_{0-\infty}$s for intravenously administered L-754,030. Cl, Vd_{ss} and $t_{½}$ values remained relatively constant over the doses studied, indicating zero-order kinetics.

Pharmacokinetic parameters for intravenously administered L-754,030 in rats

Parameter	L-754,030 (mg/kg, i.v.)		
	0.2	2	5
$AUC_{0-\infty}$ (ng•hr/ml)	201	2658	6377
Cl (ml/min/kg)	17.6	12.7	13.4
Vd_{ss} (l/kg)	3.2	2.8	2.5
$t_{½}$ (hr)	2.7	3.2	2.4

As shown in the following table, when one compares normalized AUCs [Average AUC/µmol dose (ng•hr/ml)] for L-754,030 after either L-758,298 or L-754,030 intravenous administration, the analysis suggests that 91% to 100% of the prodrug L-758,298 was converted to L-754,030.

Conversion of L-758,298 to L-754,030 in plasma of rats

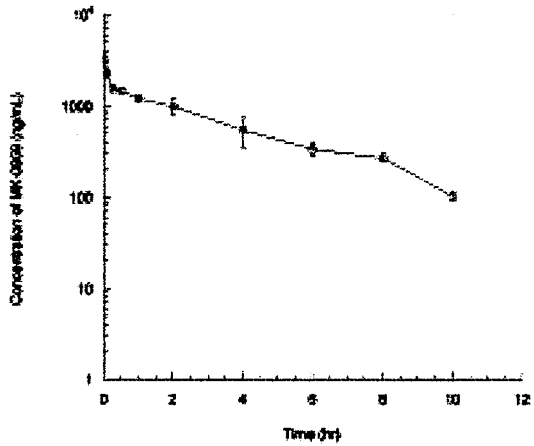
Parameter	L-758,298 (mg/kg, i.v.)		
	1	8	25
Dose (µmol/kg)	1.00	7.96	24.88
AUC of L-754,030 (ng•hr/ml)	568	6294	22392
Average AUC/µmol dose (ng•hr/ml)	568	785	900
Parameter	L-754,030 (mg/kg, i.v.)		
	0.2	2	5
Dose (µmol/kg)	0.37	3.74	9.36
AUC of L-754,030 (ng•hr/ml)	201	2658	6377
Average AUC/µmol dose (ng•hr/ml)	627	696	681
% Conversion	~91%	~100%	~100%

Pharmacokinetics and Oral Bioavailability of MK-0869 in male CD-1 Mice.

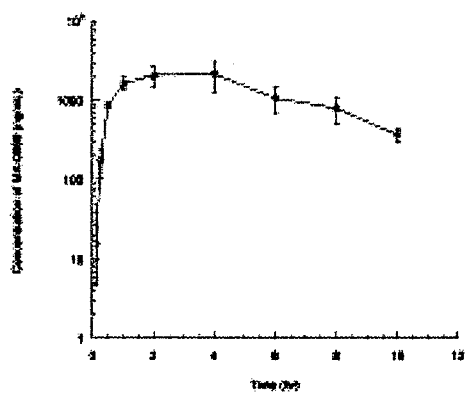
Methods: The pharmacokinetics and oral bioavailability of MK-0869 were determined after i.v (2 mg/kg) and oral (10 mg/kg) administration to male CD-1 mice. For i.v. administraion, the drug was dissolved in ethanol/propylene glycol/water and for oral administration, it was suspended 0.5% methylcellulose containing 0.02% sodium lauryl sulfate. Blood was collected by cardiac puncture from 3 animals at each time point of 2 (i.v. only), 5, 15, 30 min, and 1, 2, 4, 6, 8, 10 and 24 hrs after dosing. Plasma MK-0869 concentrations were determined by LC/MS/MS analysis.

Results: Following bolus i.v. administration of a 2 mg/kg of MK-0869 to male mice, the plasma half life was 2.6 hours, the total plasma clearance was 4.9 ml/min/kg, the volume of distribution at the steady state (Vd_{ss}) was 1.2 L/kg and the AUC was 6753.4 ng.hr./ml. The plasma drug levels after 24 hours were below the limits of detection in 2 of 3 animals. Oral bioavailability of the drug was 42.4% and the T_{max}, C_{max} and AUC values after oral dosing were 4 hrs, 2175.7 ng/ml and 14328.1 ng.hr/ml, respectively. The mean plasma MK-0869 concentrations (ng/ml) in male mice after oral and i.v. dosings are shown in the sponsor's Figures below.

Concentrations (Mean ± SD, n=3) of MK-0869 in Plasma of Male CD-1 Mice
Dosed IV at 2 mg/kg



Concentrations (Mean ± SD, n=3) of MK-0869 in Plasma of Male CD-1 Mice
Dosed Orally at 10 mg/kg

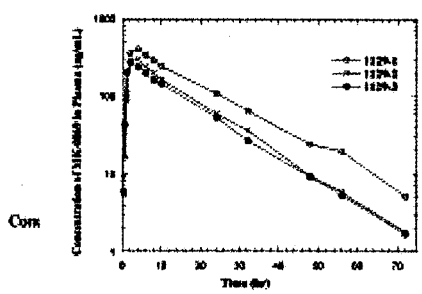


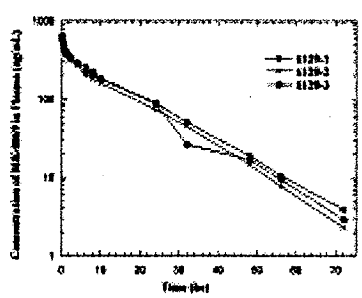
Pharmacokinetics and Oral Bioavailability of MK-0869 in Male Ferrets.

Methods: The pharmacokinetics and oral bioavailability of MK-0869 were determined in a crossover study in male ferrets after i.v. (0.5 mg/kg) and oral (1 mg/kg) dosing. There was a 2-week washout period between the i.v. and oral dosing. For i.v. administration, the drug was dissolved in ethanol/propylene glycol/water and for oral administration, the drug was suspended in 0.5% methylcellulose containing 0.02% sodium lauryl sulfate. Samples of venous blood were collected at 5 (i.v. only), 15, 30 min, and 1, 2, 4, 6, 8, 10, 24, 32, 48, 56 and 72 hours after dosing. Plasma drug concentrations were determined by LC/MS/MS analysis.

Results: Following i.v. administraion of a 0.5 mg/kg dose of MK-0869 to male ferrets, the half life ($t_{1/2}$), total plasma clearance, and the volume of distribution at the steady state (Vd_{ss}) were estimated to be 10 hr, 1.5 ml/kg/min and 1.3 L/kg, respectively. Following oral administration of a 1mg/kg dose, the mean T_{max}, C_{max} and AUC values were 3.3 hr, 326.7 ng/ml and 5150.7 ng.h/ml, respectively. The oral bioavailability of MK-0869 in male ferrets was 45.4%. The plasma MK-0869 concentrations in ferrets after i.v. and oral dosing are shown in the sponsor's Figures below.

Concentrations of MK-0869 in Plasma of Male Ferrets Dosed Orally at 1 mg/kg





1. Pharmacokinetics of intravenously administered L-754,030 and L-758,298

Animals: Male Beagle dogs (body weight range of 9.6-14.3 kg; ages were not provided by the sponsor).

Methods: L-758,298 was dissolved in a solution of lactose (50 mg/ml), potassium chloride (1.38 mg/ml), citric acid monohydrate (0.85 mg/ml) and sodium chloride (4 mg/ml) (pH 7.0); dosing volumes were 1, 5 and 10 mg/ml of L-758,298, respectively. L-754,030 was prepared in a solution of ethanol:propylene glycol:water (10:60:30,v/v/v); dosing volume was 0.5, 1 and 2 mg/ml of L-754,030, respectively.

Dogs (3 per dose) were intravenously administered either L-758,298 (0.5, 2 and 32 mg/kg) or L-754,030 (0.2, 0.5 and 2 mg/kg) via the jugular vein. Blood samples were withdrawn from the femoral vein at 0.042, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 24, 30, 48, and 72 hr after dosing. Plasma concentrations of L-754,030 and L-758,298 were determined by a LC/MS/MS assay.

Results: As shown in the following table, there were dose-related increases in $AUC_{0-\infty}$s for intravenously administered L-754,030. Cl, Vd_{ss} and $t_{½}$ values remained relatively constant over the doses studied, indicating zero-order kinetics.

Pharmacokinetic parameters for intravenously administered L-754,030 in dogs

Parameter	L-754,030 (mg/kg, i.v.)		
	0.2	0.5	2
$AUC_{0-\infty}$ (ng•hr/ml)	1266	3786	37613
Cl (ml/min/kg)	2.6	2.3	0.9
Vd_{ss} (l/kg)	1.0	1.1	0.9
$t_{½}$ (hr)	5.7	7.3	ND

As shown in the following table, when one compares normalized AUCs [Average AUC/µmol dose (ng•hr/ml)] for L-754,030 after either L-758,298 or L-754,030 intravenous administration, the analysis suggests that 64% to 86% of the prodrug L-758,298 was converted to L-754,030.

Conversion of L-758,298 to L-754,030 in plasma of dogs

Parameter	L-758,298 (mg/kg, i.v.)		
	0.5	2	32
Dose (µmol/kg)	0.5	1.99	31.84
AUC of L-754,030 (ng•hr/ml)	1093	8926	373363
Average AUC/µmol dose (ng•hr/ml)	2186	4697	11726
Parameter	L-754,030 (mg/kg, i.v.)		
	0.2	0.5	2
Dose (µmol/kg)	0.37	0.94	3.74
AUC of L-754,030 (ng•hr/ml)	1266	3786	37613
Average AUC/µmol dose (ng•hr/ml)	3421	4028	10057
% Conversion	~64%	~86%	~86%

Pharmacokinetics of L-000758298 in Male and Female Sprague-Dawley Rats Using the New Higher Concentration (25 mg/ml) Formulation.

Methods: The pharmacokinetics of L-000758298 (pro-drug) and the pharmacologically active entity, aprepitant (L-000754030), were studied in male and female Sprague-Dawley rats following i.v. (bolus) administration of 5.0, 7.5 and 10 mg/kg doses of L-000758298. L-000758298 (N-methyl-D-glucamine) was dissolved in 0.9% saline containing 2 mM EDTA and the solution was sterilized by filtration. The doses were administered via a cannula inserted into the femoral vein. Blood samples for determination of plasma concentrations of L-000758298 and aprepitant were collected at 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 hours after administration of the doses. Plasma concentrations of L-000758298 and aprepitant were determined by an LC/MS/MS method.

Results: L-000785298 was rapidly converted to its active metabolite, aprepitant, in rats following single intravenous administration of 5.0, 7.5 and 10 mg/kg doses. The maximum plasma concentrations of aprepitant were achieved at the first sampling point (0.033 hr) and were detectable up to 24 hours after dosing. The mean plasma $AUC_{(0-\alpha)}$ values of aprepitant increased in a dose-proportional manner in both male and female rats, as the dose was increased from 5 to 10 mg/kg. The pharmacokinetic parameters of L-000758298 and aprepitant in male and female rats following i.v. administration of 5, 7.5 and 10 mg/kg doses of L-000758298 are shown in the Table below.

Summary of Pharmacokinetic Parameters for Aprepitant and L-000758298 in Male and Female SD Rats Administered an IV Bolus Dose of L-000758298 at 5, 7.5, and 10 mg/kg[a]

Dose (mg/kg)	Compound	$AUC_{(0\text{-}24\ hr)}$ (ng•hr/mL)	$AUC_{(0\text{-}\infty)}$ (ng•hr/mL)	C_{max} (ng/mL)[b]	T_{max} (hr)
5	Aprepitant	5280	5290	3780	0.033
(male)	L-000758298	NC[c]	NC	975	0.033
7.5	Aprepitant	9980 ± 1730	10000 ± 1740	5890 ± 1020	0.033
(male)	L-000758298	NC	NC	3460 ± 899	0.033
10	Aprepitant	16600 ± 2610	16600 ± 2590	6870 ± 1770	0.033
(male)	L-000758298	NC	NC	3070 ± 3200	0.033
5	Aprepitant	16800 ± 2050	17000 ± 1840	4750 ± 881	0.033
(female)	L-000758298	NC	NC	840 ± 386	0.033
7.5	Aprepitant	21900 ± 5950[d]	24100 ± 2760	6800 ± 1370	0.033-0.083
(female)	L-000758298	NC	NC	2720 ± 881	0.033
10	Aprepitant	33000	33100	11700	0.033
(female)	L-000758298	NC	NC	4330	0.033

[a] Male and female SD rats were dosed intravenously with a 25 mg/mL solution of L-000758298 formulated in 0.9% saline and 2 mM EDTA (pH=8.0 adjusted with 1 M NaOH). Male (and female) rats in the 5, 7.5, and 10 mg/kg dose groups received average doses of 4.68 (6.10), 7.69 (7.59), and 10.7 (10.8) mg/kg, respectively. Concentrations of aprepitant and L-000758298 in sodium vanadate (final concentration=10 mM) treated plasma were determined by LC-MS/MS following protein precipitation. Mean (n=2) or mean ± standard deviation (n=3) values are listed.

[b] C_{max} for L-000758298 represents the concentration at the earliest time point evaluated (0.033 hr).

[c] NC= not calculated.

[d] AUC $_{(0\text{-}8hr)}$ was used in one of the rats because the animal died after 8 hr.

Pharmacokinetics of L-000758298 in Male Beagle Dogs Using the New Higher Concentration (25 mg/ml) Formulation.

Methods: The pharmacokinetics of L-000758298 (pro-drug) and the pharmacologically active entity, aprepitant (L-000754030), were studied in male beagle dogs (9.0-11.0 kg) following i.v. (bolus) administration of 4.0 and 6.0 mg/kg doses of L-000758298. L-000758298 (N-methyl-D-glucamine) was dissolved in 0.9% saline containing 2 mM EDTA and the solution was sterilized by filtration. The doses were administered via a cannula inserted into the cephalic vein. Blood samples for determination of plasma concentrations of L-000758298 and aprepitant were collected at 0.033, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 hours after administration of the doses. Plasma concentrations of L-000758298 and aprepitant were determined by an LC/MS/MS method.

Results: L-000785298 was rapidly converted to its active metabolite, aprepitant, in dogs following single intravenous administration of 4.0 and 6.0 mg/kg doses. The maximum plasma concentrations of aprepitant were achieved at the first sampling point (0.033 hr) and were detectable up to 54 to 72 hours post-dose. The mean plasma $AUC_{(0\text{-}24\ hr)}$ values of aprepitant was similar at 4 (54,800 ng.hr/ml) and 6 (50,800 ng.h/ml) mg/kg doses. The mean plasma AUC values for L-000758298 were also similar at 4 and 6 mg/kg doses. The pharmacokinetic parameters of L-000758298 and aprepitant in male and female dogs following i.v. administration of 4 and 6 mg/kg doses of L-000758298 are shown in the Table below.

Summary of Pharmacokinetic Parameters for Aprepitant and L-000758298 in Male Beagle Dogs Administered an IV Bolus Dose of L-000758298 at 4 and 6 mg/kg [a]

Dose (mg/kg)	Compound	$AUC_{(0\text{-}24\ hr)}$ (ng•hr/mL)	$AUC_{(0\text{-}72\ hr)}$ (ng•hr/mL)	C_{max} (ng/mL)[b]	T_{max} (hr)
4	Aprepitant	54800 ± 9340	74800 ± 19000	10300 ± 825	0.033
	L-000758298	NC[c]	NC	17400 ± 2720	0.033
6	Aprepitant	50800 ± 8700	70100 ± 15200	9410 ± 774	0.033
	L-000758298	NC	NC	14400 ± 1700	0.033

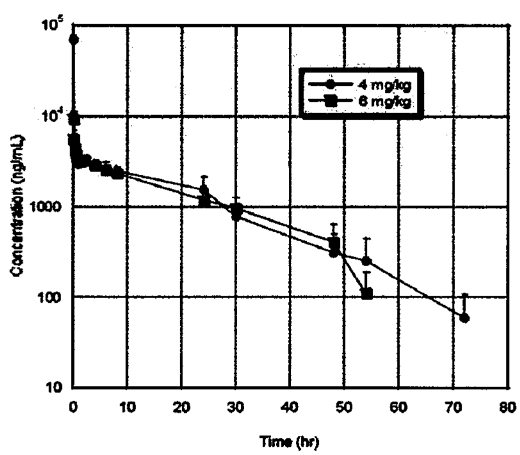
[a] Male Beagle dogs were dosed intravenously with a 25 mg/mL solution of L-000758298 formulated in 0.9% saline and 2 mM EDTA (pH=8.0 adjusted with 1 M NaOH). Concentrations of aprepitant and L-000758298 in sodium vanadate (final concentration=10 mM) treated plasma were determined by LC-MS/MS following protein precipitation. Mean ± standard deviation values from n=3 are listed.

[b] C_{max} for L-000758298 represents the concentration at the earliest time point evaluated (0.033 hr).

[c] NC= not calculated.

The mean plasma concentrations of L-000758298 and aprepitant in dogs administered 4 and 6 mg/kg i.v. doses are shown in the Figure below.

Concentrations of Aprepitant in Plasma from Dogs Dosed with Intravenous Bolus Doses of L-000758298 at 4 and 6 mg/kg[a]



2.6.4.4 Distribution

Tissue Distribution and Excretion of Radioactivity Following Single Intravenous Dose of [^{14}C]L-758,298 in Male Rats

Methods: The excretion and tissue distribution of radioactivity was assessed in male Sprague-Dawley rats following administration of a single 3 mg/kg (1 ml/kg) intravenous dose of [^{14}C]L-758,298. Three animals/time point were sacrificed at 5 and 30 minutes and 4, 24 and 120 hours post-dose. Blood, adrenal gland, bone, bone marrow, brain, eyes, fat, heart, kidneys, large intestine, large intestinal contents and wash, liver, lungs, lymph nodes (cervical and mesenteric), muscle, pancreas, pituitary, prostate, skin, small intestine, small intestinal contents and wash, spleen, stomach, stomach contents and wash, testes, thymus, thyroid/parathyroid and urinary bladder were collected. Urine and feces were collected from 3 animals at 24 hour intervals through 120 hours post-dose. After the collection of the last excreta, cages were washed and the radioactivity measured by liquid scintillation counting. Blood was collected at 24 hours post-dose prior to sacrifice for analysis of blood and plasma directly by liquid scintillation counting.

Results: Maximum mean tissue concentrations of [^{14}C]L-758,298-derived radioactivity were observed at 5 minutes through 4 hours post-dose in all tissues. The adrenal gland, liver, kidneys, and thyroid/parathyroid reached a maximum radioactivity concentration at 5 minutes, and had the highest mean concentration of radioactivity at this time point with values of 20.9, 15.9, 11.9 and 11.0 µg equivalents/g, respectively. Maximum concentrations of the radioactivity in blood (0.517 µg equivalents/g) and plasma (0.799 µg equivalents/g) were reached in 5 minutes post-dose, and declined steadily throughout the 120 hours study period. The radioactivity penetrated the blood-brain barrier over time. Radioactivity concentration in the fat, large intestinal contents and wash, testes and brain reached maximum at 4 hours post-dose with mean values of 1.59, 0.486, 0.426 and 0.315 µg equivalents/g, respectively. The radioactivity concentrations in all tissues declined with time. The concentrations in all tissues were lower than 1.33 µg equivalents/g (except liver) at 24 hr post-dose. The mean radioactivity concentrations in the blood, plasma and tissues at different time points following i.v. administration of a 2 mg/kg dose are shown in the Tables below.

Table 2. Mean Concentration of Radioactivity in Blood, Plasma, and Tissues at Specified Times Postdose for Male Rats (Group 1) Following a Single Intravenous Dose of [^{14}C]L-758,298 (2 mg/kg)

	µg Equivalents [^{14}C]L-758,298/g					
	5 Minutes		30 Minutes		4 Hours	
Matrix	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	20.9	2.73	7.00	0.560	3.84	0.256
Blood	0.517	0.043	0.504	0.025	0.414	0.021
Bone (both femurs)	1.31	0.058	0.924	0.038	0.574	0.043
Bone marrow (from both femurs)	3.99	0.330	2.48	0.087	1.34	0.116
Brain	0.119	0.005	0.193	0.019	0.315	0.022
Carcass (residual)	1.15	0.130	NS	NA	NS	NA
Eyes	0.482	0.078	0.338	0.020	0.309	0.028
Fat (reproductive area)	0.285	0.038	0.795	0.023	1.59	0.106
Heart	5.60	0.259	3.76	0.211	1.79	0.236
Kidneys	11.9	2.04	3.36	0.402	1.91	1.44
Large intestine	2.38	1.59	1.55	0.126	1.38	0.044
Large intestinal contents and wash	0.020	0.001	0.026	0.007	0.486	0.254
Liver	15.9	1.74	11.1	1.94	8.20	0.262
Lungs	2.87	0.513	1.90	0.346	2.01	0.577
Lymph nodes (cervical)	0.755	0.009	1.26	0.015	1.43	0.037
Lymph nodes (mesenteric)	0.841	0.059	1.32	0.059	1.55	0.067
Muscle (thigh)	1.07	0.064	1.33	0.061	1.10	0.084
Pancreas	4.72	0.908	4.36	0.654	2.52	0.278
Pituitary	1.78	0.255	2.36	0.233	2.33	0.339
Plasma	0.799	0.039	0.775	0.066	0.709	0.030
Prostate	1.01	0.134	1.67	0.120	1.62	0.147
Skin (dorsal, shaved)	0.623	0.105	0.830	0.008	0.949	0.086
Small intestine	1.93	1.10	3.40	0.381	3.47	0.612
Small intestinal contents and wash	0.680	0.341	1.21	0.041	3.17	0.363
Spleen	1.75	0.237	1.83	0.249	1.04	0.169
Stomach	2.96	0.247	2.42	0.371	1.68	0.139
Stomach contents and wash	ND	NA	0.405	0.660	0.153	0.068
Testes	0.073	0.010	0.127	0.009	0.426	0.013
Thymus	0.776	0.109	1.04	0.025	1.31	0.068
Thyroid/parathyroid	11.0	0.863	2.84	0.189	2.02	0.215
Urinary bladder	0.756	0.025	0.964	0.079	1.11	0.130

NA Not applicable.
ND Not detectable.
NS No sample.
SD Standard deviation.

Table 2 (Continued). Mean Concentration of Radioactivity in Blood, Plasma, and Tissues at Specified Times Postdose for Male Rats (Group 1) Following a Single Intravenous Dose of [^{14}C]L-758,298 (2 mg/kg)

	µg Equivalents [^{14}C]L-758,298/g			
	24 Hours		120 Hours	
Matrix	Mean	SD	Mean	SD
Adrenal glands	0.640	0.127	NS	NA
Blood	0.093	0.014	0.044	0.034
Bone (both femurs)	0.105	0.008	NS	NA
Bone marrow (from both femurs)	0.286	0.054	NS	NA
Brain	0.087	0.011	NS	NA
Eyes	0.060	0.014	NS	NA
Fat (reproductive area)	0.976	0.235	NS	NA
Heart	0.316	0.086	NS	NA
Kidneys	0.931	0.952	NS	NA
Large intestine	0.443	0.053	NS	NA
Large intestinal contents and wash	0.467	0.050	NS	NA
Liver	1.33	0.208	NS	NA
Lungs	0.249	0.028	NS	NA
Lymph nodes (cervical)	0.297	0.075	NS	NA
Lymph nodes (mesenteric)	0.364	0.076	NS	NA
Muscle (thigh)	0.152	0.028	NS	NA
Pancreas	0.403	0.111	NS	NA
Pituitary	ND	NA	NS	NA
Plasma	0.124	0.018	0.010	0.002
Prostate	0.330	0.041	NS	NA
Skin (dorsal, shaved)	0.199	0.053	NS	NA
Small intestine	0.508	0.124	NS	NA
Small intestinal contents and wash	0.609	0.308	NS	NA
Spleen	0.263	0.163	NS	NA
Stomach	0.263	0.060	NS	NA
Stomach contents and wash	0.096	0.100	NS	NA
Testes	0.164	0.021	NS	NA
Thymus	0.211	0.038	NS	NA
Thyroid/parathyroid	0.380	0.096	NS	NA
Urinary bladder	0.535	0.012	NS	NA

NA Not applicable.
ND Not detectable.
NS No sample.
SD Standard deviation.

Approximately 94%, 83%, 83% and 26% of the administered radioactive dose was found in the collected tissues and residual carcases at 5 minutes, 30 minutes, 4 hours and 24 hours post-dose, respectively.
The main route of excretion of [^{14}C]L-758,298 derived radioactivity following i.v. administration to male rats was via the feces. A mean of 58.3% was excreted via the feces and 36.1% was excreted in the urine by 120 hours post-dose. The mean 0-120 hr recovery of radioactivity in tissues and excreta, including cage washes and wipes, was 95.2%.

The percent of the radioactive dose in the urine, feces, cage wash and cage wipe at different time intervals following dosing are shown in the Table below.

Table 13. Percent of Radioactive Dose in Urine, Feces, Cage Wash, and Cage Wipe at Specified Intervals Postdose for Male Rats (Group 1) Following a Single Intravenous Dose of [^{14}C]L-758,298 (2 mg/kg)

Collection Interval (Hours)	Percent of Radioactive Dose				
	Animal Number				
	C21023	C21029	C21025	Mean	SD
		Urine			
0-24	27.9	27.5	30.4	28.6	1.56
24-48	4.86	5.16	4.84	4.96	0.18
48-72	1.61	1.41	1.54	1.52	0.10
72-96	0.66	0.73	0.73	0.70	0.04
96-120	0.34	0.32	0.33	0.33	0.01
Subtotal	35.3	35.2	37.8	36.1	1.50
		Feces			
0-24	43.7	38.8	34.9	39.1	4.39
24-48	11.9	13.3	13.2	12.8	0.76
48-72	4.09	4.36	4.30	4.25	0.14
72-96	1.64	1.38	1.30	1.44	0.17
96-120	0.68	0.59	0.75	0.67	0.08
Subtotal	62.0	58.4	54.5	58.3	3.76
		Cage Wash and Cage Wipe			
120[a]	0.29	0.33	0.50	0.37	0.11
120[b]	ND	0.14	0.30	0.15	0.15
Total	97.6	94.0	93.1	94.9	2.39

ND Not detectable.
SD Standard deviation.

a Cage wash.
b Cage wipe.

Mass Balance, Tissue Distribution and Metabolism Studies of [^{14}C]MK-0869 in Rats.

Methods: The mass balance, tissue distribution and biliary excretion of [^{14}C]MK-0869 were studied in adult male SD rats after i.v. administration of a 2 mg/kg dose, and oral administration of 2, 5 or 100 mg/kg doses. The metabolism of [^{14}C]MK-0869 was studied in plasma, urine, bile and feces collected from these animals after i.v. or oral dosing. Urine and fecal samples were collected at intervals of 0-24, 24-48, 48-72, 72-96 and 96-120 hours. Samples of blood were collected by cardiac puncture at 5 min, 30 min, 4 hr, and 24 hr post-dose (3 animals at each time point). The presence of the metabolites in these biological matrices was confirmed by LC-MS/MS analyses by comparison with authentic standard metabolites.

Results: Following i.v. and oral administration of a 2 mg/kg dose of [^{14}C]MK-0869 to rats, fecal excretion accounted for an average of 53.8% and 57.8% of the radioactivity, respectively in 120 hours. The radioactivity recovered in the urine for 120 hours following i.v. and oral dosing accounted for 33.7% and 29.5% of the radioactivity, suggesting that biliary route is the major route of excretion in rats.

Maximum mean tissue concentrations of MK-0869-derived radioactivity were observed from 5 minutes through 4 hours after dosing. The adrenal glands, liver, lungs and the heart had the highest mean concentrations of 15.8, 13.0, 12.0 and 11.6 µg equivalents/g of tissue, respectively. The lowest concentrations were determined in the testes, brain and fat, and had 0.071, 0.136 and 0.286 µg equivalents/g of tissue, respectively. Approximately 93, 82, 81 and 24% of the administered radioactive dose was recovered in the tissues and the residual carcass at 5 min, 30 min, 4 hr and 24 hr post-dose, respectively. The tissue concentrations of the radioactivity at different times after dosing are shown in the sponsor's Table below.

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Mean (n=3) Tissue Concentrations of Radioactivity in Rats
Following IV Administration of [[14]C]MK-0869 at 2 mg/kg

	MK-0869 Radioequivalents (µg/g)							
	5 Min		30 Min		4 Hours		24 Hours	
Tissue	Mean	SD[a]	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	15.8	4.65	7.38	0.652	4.07	0.370	0.637	0.509
Blood	0.807	0.101	0.504	0.026	0.422	0.011	0.096	0.003
Bone (both femurs)	0.857	0.047	0.884	0.044	0.658	0.084	0.115	0.014
Bone marrow (from both femurs)	1.48	0.119	2.01	0.037	1.35	0.125	0.260	0.005
Brain	0.136	0.019	0.242	0.013	0.471	0.008	0.151	0.019
Carcass (residual)	1.40	0.046	NS[b]	NA[c]	NS	NA	0.261	0.076
Eyes	0.441	0.121	0.355	0.038	0.265	0.016	0.052	0.026
Fat (reproductive area)	0.286	0.015	0.910	0.021	2.18	0.268	1.59	0.071
Heart	11.6	2.71	4.79	0.689	2.29	0.211	0.421	0.121
Kidneys	7.17	0.575	5.07	0.214	3.10	0.308	0.618	0.123
Large intestine	0.853	0.047	1.43	0.106	1.54	0.160	0.415	0.012
Large intestinal contents and wash	0.010	0.009	0.031	0.018	0.188	0.091	0.474	0.114
Liver	13.0	1.81	11.4	0.162	9.15	0.521	1.55	0.225
Lungs	12.0	2.36	4.18	0.308	3.51	0.166	0.625	0.050
Lymph nodes (cervical)	0.734	0.172	1.40	0.178	1.45	0.131	0.312	0.029
Lymph nodes (mesenteric)	0.718	0.138	1.24	0.129	1.38	0.149	0.446	0.034
Muscle (thigh)	1.62	0.358	1.77	0.240	1.22	0.086	0.140	0.015
Pancreas	4.12	0.451	5.05	0.345	2.98	0.230	0.479	0.045
Pituitary	4.09	1.99	4.09	1.65	2.29	0.361	0.394	0.002
Plasma	1.18	0.148	0.768	0.043	0.097	0.030	0.120	0.006
Prostate	0.654	0.100	1.22	0.207	1.88	0.350	0.331	0.033
Skin (dorsal, shaved)	0.348	0.089	0.679	0.191	0.871	0.130	0.185	0.012
Small intestine	1.50	0.136	2.41	0.211	5.72	0.550	0.460	0.035
Small intestinal contents and wash	0.168	0.046	1.18	0.578	3.07	0.388	0.444	0.065
Spleen	2.39	0.382	2.10	0.081	1.50	0.060	0.386	0.040
Stomach	2.10	0.117	2.39	0.121	1.68	0.178	0.281	0.021
Stomach contents and wash	0.004	0.006	0.071	0.060	0.127	0.030	0.036	0.046
Testes	0.071	0.003	0.122	0.005	0.430	0.012	0.216	0.022
Thymus	1.14	0.148	1.34	0.035	1.50	0.089	0.362	0.037
Thyroid/parathyroid	9.26	2.59	3.65	0.222	2.59	0.228	0.534	0.086
Urinary bladder	0.504	0.038	0.790	0.038	1.44	0.572	0.679	0.072

[a]SD: Standard deviation; NA: Not applicable; NS: No sample.

The biliary excretion was studied in bile duct cannulated rats following i.v. administration of a 2 mg/kg and oral administration of a 5 mg/kg dose of [[14]C]MK-0869. The absorption of the radioactivity by the oral route was approximately 43%, and about 31% and 12% of the dose was excreted in the bile and urine, respectively. The unchanged drug in the bile accounted for ~7% and 5% of the dose, and a major polar metabolite (glucuronide of MK-0869) accounted for ~17% and 18% of the dose, after i.v. and oral doses, respectively.

In the rat plasma, several nonpolar (L-755446, L-809861, L-829674, L-826678, L-809771), polar (L-829615, L-819617) and very polar (L-596064, L-294569, L-770787) metabolites were identified. The structures of these metabolites are shown in the sponsor's Figure below.

1. Stability of L-758,298 in blood from rat, dog and human

In order to establish assay methods for L-758,298 in plasma, the stability of L-758,298 was studied in blood from rat, dog and human.

Methods: L-758,298 (1 and 10 μg/ml) was incubated in fresh heparinized blood at 37° for 0-120 min. After incubation, vanadate (5 mM) was added immediately and the samples were kept on ice to minimize any *ex vivo* hydrolysis of L-758,298. Blood was centrifuged and 0.2 ml aliquots of plasma were mixed with internal standard, processed by solid-phase extraction and analyzed for L-758,298 and L-754,030 by LC/MS/MS.

Results: As shown in the following table, the stability of L-758,298 was greater in human plasma (< 15% conversion during 2 hrs) than in dog or rat plasma. The stability was greater in dog plasma (30-30 % conversion during 2 hrs) than in rat plasma (half-life of about 30 min).

Pharmacokinetics and Brain/Plasma Distribution of MK-0869 in CD-1 Mice.

Methods: The pharmacokinetics and brain/plasma distribution of MK-0869 were investigated in the mdr 1a (+/+) CF-1 mice, capable of P-glycoprotein (Pgp) expression and those that are deficient in mdr 1a (-/-), to assess the role of Pgp in the disposition of MK-0869 after i.v. and oral administration. For i.v. administration, the drug was dissolved in control mouse plasma and injected into the tail vein at a dose of 2.35 mg/kg (0.15 ml/mouse). For oral administration, the drug was dissolved in 20% hydroxypropyl β-cyclodextrin and administered by oral gavage at a dose of 5 mg/kg (2.5 ml/kg). Ondansetron and dexamethasone, two antiemetic agents, and known Pgp substrates were administered to determine the potential Pgp-mediated drug-drug interactions. Dexamethasone was given at an i.v. dose of 1 mg/kg and ondansetron was given at an oral dose of 0.5 mg/kg. The concentrations of MK-0869 and its metabolite, L-755446 in the plasma and brain extracts were determined by LC-MS/MS.

Results: Following i.v. or oral administration of MK-0869 to mdr 1a (+/+) and mdr 1a (-/-) mice, the bioavailability of the drug was similar in both groups. However, the peak plasma concentration was slightly higher and the time-to-peak concentration was slightly delayed in the mdr 1a (+/+) mice. There was rapid distribution of MK-0869 into the brain of both groups of mice. The peak plasma concentrations were achieved at 0.5 and 2 hours in mdr 1a (+/+) and mdr 1a (-/-) mice, respectively. The concentrations of MK-0869 in the brain of mdr 1a (-/-) mice were higher than that of mdr 1a (+/+) mice after either i.v. or oral dosing. The ratio of brain to plasma AUC values (AUC_{brain}/AUC_p) in the mdr 1a (-/-) mice was about 13-fold higher after i.v dosing and about 19-fold higher after oral dosing, as compared with that of mdr 1a (+/+) mice, suggesting that MK-0869 is a substrate for mdr 1a P-glycoprotein. The concentrations of the metabolite, L-755446 in the plasma of both groups of mice were below the limits of detection, and the brain concentrations were similar in both groups (5-20 ng/g of tissue), suggesting that L-755446 may not be a substrate for mdr 1a P-glycoprotein. The plasma and brain concentrations and the pharmacokinetic parameters of MK-0869 in two groups of CF-1 mice are shown in the sponsor's Tables below.

Table 1. Plasma Concentrations (ng/mL) and Pharmacokinetic Parameters of MK-0869 in Pgp (+/+) or (-/-) CF-1 Mice Following a 2.35 mg/kg IV Dose of MK-0869 (Mean ± SD, n=3 per time point) (PDM 1444)

Time (hr)	Pgp (+/+)	Pgp (-/-)
0.083	1173 ± 159.2	1528 ± 94.1
0.5	755.4 ± 42.1	624.1[b]
2	429.7 ± 37.1	267.0[b]
6	152.0 ± 19.7	244.6 ± 97.9
8	77.2 ± 14.3	26.3[b]
$AUC_{p(0-8hr)}$ (ng·hr/mL)[a]	2433	2310
CL_p (mL/min/kg)[a]	14.4	16.5
Vd_{ss} (L/kg)[a]	2.6	2.2
t½ (hr)[a]	2.5	1.7

[a]Parameters were calculated based on mean plasma concentration data.
[b]For this time point n=2.
Notebook/page: 15183/121 (Feb-Jun-1998)

Table 2. Brain Concentrations of MK-0869 and L-755446 in Pgp (+/+) or (-/-) CF-1 Mice Following a 2.35 mg/kg IV Dose of MK-0869 (Mean ± SD, n=3 per time point) (PDM1444)

	Brain (ng/g Tissue)			
	MK-0869		L-755446	
Time (hr)	Pgp (+/+)	Pgp (-/-)	Pgp (+/+)	Pgp (-/-)
0.083	56.1 ± 5.52	349.5 ± 8.96	6.49 ± 1.12	6.81 ± 0.69
0.5	92.5 ± 24.7	678.8 ± 25.0	18.8 ± 2.93	18.0 ± 0.41
2	63.0 ± 7.52	798.5 ± 147.5	19.1 ± 0.58	13.9 ± 3.03
6	37.8 ± 3.32	638.3 ± 284.1	12.2 ± 0.84	8.67 ± 2.81
8	19.3 ± 1.84	205.2 ± 76.5	8.43 ± 1.73	5.03 ± 1.17
$AUC_{brain(0-8hr)}$ (ng·hr/g)[a]	408.6	5054	117.2	88.2

When MK-0869 was co-administered orally with ondansetron, the brain MK-0869 concentrations or the brain to plasma concentration ratios were not affected by ondansetron in mdr 1a (+/+) mice. Similarly, when MK-0869 was co-administered with intravenous dexamethasone, the brain concentrations or the brain to plasma concentration ratios for MK-0869 were unchanged in these mice. The plasma and brain concentrations and AUC values for MK-0869 following co-administration with ondansetron or dexamethasone are shown in the sponsor's Table below.

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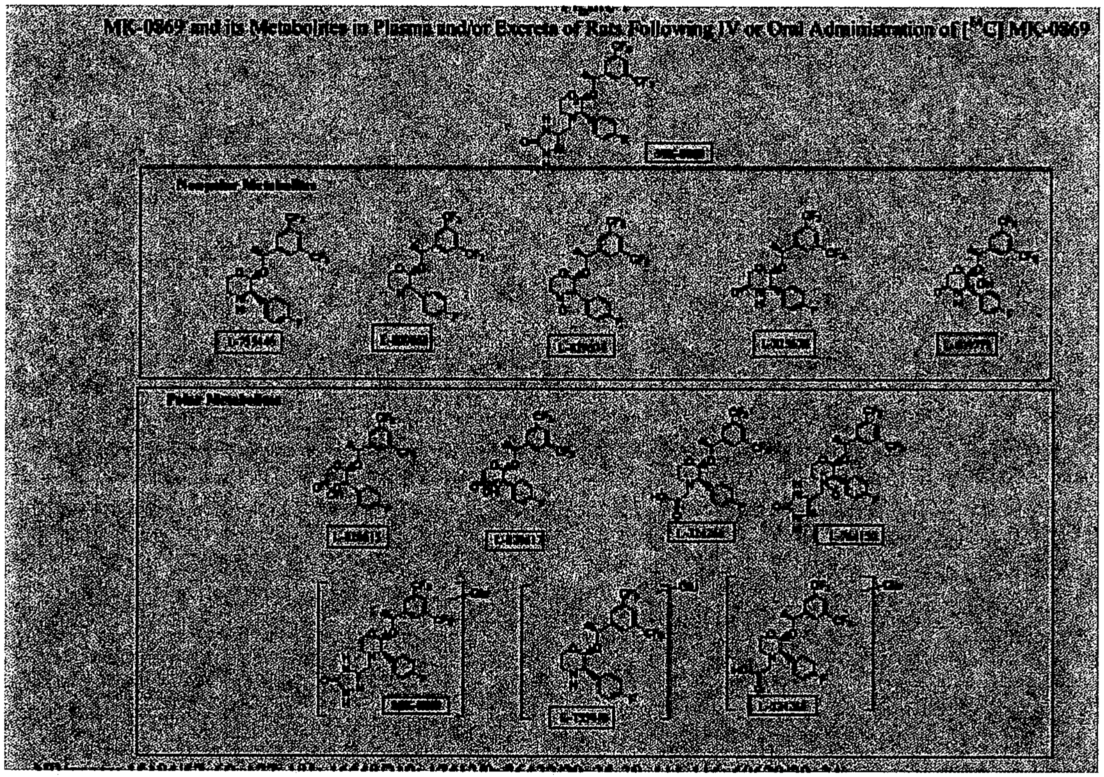

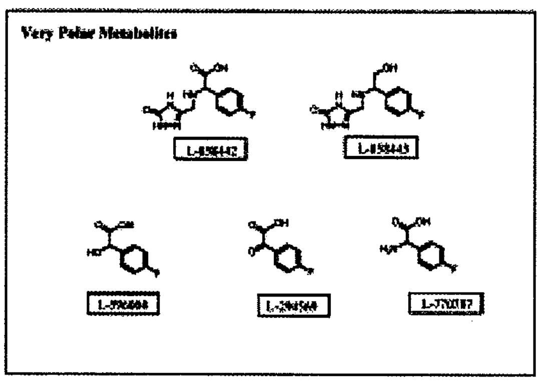

In the rat feces, the major radioactive peak was identified as [^{14}C]MK-0869. Several nonpolar (L-755446, L-809861, L-825678, L-829674, L-809771) and polar (L-829615 and L-829617) metabolites, previously identified in the rat plasma, were also detected in the feces. In addition, a polar metabolite, L-324261 and the N-oxide of MK-0869, L-764120 was detected in the fecal extracts. In the bile, in addition to the metabolites detected in plasma, four polar metabolites (L-324261, L-809861, L-596064 and L-294569) were detected in animals receiving the i.v. dose. In the urine, the presence of four very polar metabolites was confirmed by LC-MS/MS; the major metabolites in urine were L-596064 and L-294569.

2. Plasma protein binding of L-754,030 in plasma from rat, dog and human

Methods: [Ethyl-1,2-^{3}H]L-754,030 in 20 µl of acetonitrile and methanol (9:1, v/v) and various concentrations of non-radioactive L-754,030 were mixed with 4 ml of pooled plasma from rats, dogs and humans, respectively. Final L-754,030 concentrations of 0.01, 0.1, 1 and 10 µg/ml were studied. Samples were mixed and incubated at 37° for 30 min. Radioactivity was determined by scintillation counters.

Results: At all concentrations studied (0.0-10 µg/ml), >98% of L-754,030 was bound in plasma from rat, dog and human.

Rats, Dogs and Humans

1. Distribution of L-754,030 in plasma and red blood cells from rat, dog and human

Methods: Ten µl of various concentrations of [ethyl-1,2-^{3}H] L-754,030 (approximately 100,000 dpm) in acetonitrile and methanol (9:1,v/v) solution was added to 1 ml aliquots of fresh heparinized blood samples from rat, dog and human for final [ethyl-1,2-^{3}H]L-754,030 concentrations of 0.01, 0.1, 1 and 10 µg/ml. The samples were incubated at 37° for 30 min. Radioactivity was determined in scintillation counters. Ratios of radioactivity in whole blood to plasma were calculated. Hematocrits were also determined and expressed as %RBC in whole blood.

Results: The ratio of radioactivity for [ethyl-1,2-^{3}H]L-754,030 in red blood cells to plasma was 0.75, 0.55 and 0.61 for rat, dog and human, respectively, indicating that [ethyl-1,2-^{3}H]L-754,030 was preferentially distributed in red blood cells. The ratios did not vary over [ethyl-1,2-^{3}H]L-754,030 concentrations of 0.01 to 10 µg/ml. Hematocrits (% RBC in whole blood) were 0.48, 0.57 and 0.45 for rat, dog and human, respectively.

Table 3. Plasma Concentrations of MK-0869 in Pgp (+/+) and (-/-) CF-1 Mice Following a 5 mg/kg Oral Dose of MK-0869, in the Presence or Absence of 0.5 mg/kg Ondansetron (OND) (Mean ± SD, n=3 per time point) (PDM 1682)

Time (hr)	Plasma (ng/mL)			
	Pgp (+/+)	Pgp (+/+) with OND	Pgp (-/-)	Pgp (-/-) with OND
0.5	122.7 ± 46.9	165.8 ± 45.0	208.6 ± 48.6	255.0 ± 16.3
2	442.4 ± 74.8	612.0 ± 146.2	566.2 ± 82.6	793.4 ± 137.8
4	932.7 ± 61.5	573.8 ± 24.3	432.5 ± 209.0	366.8 ± 69.1
6	309.5 ± 37.8	414.5 ± 179.2	351.2 ± 63.1	284.4 ± 61.0
10	157.5 ± 18.8	158.3 ± 17.8	121.2 ± 27.3	168.3 ± 43.7
$AUC_{p(0-10hr)}$ (ng·hr/mL)[a]	4005.8	3944.5	3360.5	3566.9
F(%)[b]	77%	76%	68%	73%

[a]Based on mean plasma concentration data.
[b]Bioavailability (F) was calculated based on the mean AUC value (dose normalized) following the IV dose (Table 1).
Notebook/page: 15183/239-240 (Sep-Oct-1998)

Table 4. Brain Concentrations of MK-0869 in Pgp (+/+) and (-/-) CF-1 Mice Following a 5 mg/kg Oral Dose of MK-0869, in the Presence or Absence of 0.5 mg/kg Ondansetron (OND) (Mean ± SD, n=3 per time point) (PDM 1682)

Time (hr)	Brain (ng/g tissue)			
	Pgp (+/+)	Pgp (+/+) with OND	Pgp (-/-)	Pgp (-/-) with OND
0.5	4.8[b]	3.7 ± 1.1	26.6 ± 8.2	33.7 ± 5.0
2	13.7 ± 3.8	20.4 ± 4.0	192.0 ± 45.4	309.1 ± 82.4
4	36.6 ± 1.2	28.8 ± 0.7	346.5 ± 79.6	353.9 ± 20.3
6	28.7 ± 1.8	29.1 ± 9.3	598.6 ± 184.0	475.4 ± 64.5
10	11.8 ± 2.8	13.5 ± 2.4	283.8 ± 35.0	334.1 ± 73.1
$AUC_{brain(0-10hr)}$ (ng·hr/g)[a]	211.7	211.3	3419	3377

[a]Based on mean brain concentration data.
[b]For this time point n=2.

Table 5. Plasma and Brain Concentrations of MK-0869 in Pgp (+/+) CF-1 Mice Following a 0.5 mg/kg IV Dose of MK-0869, in the Presence or Absence of 1 mg/kg IV Dexamethasone (DEX) (Mean ± SD, n=3 per time point) (PDM 1773)

Time (hr)	Plasma (ng/mL)		Brain (ng/g tissue)[a]	
	Pgp (+/+)	Pgp (+/+) with DEX	Pgp (+/+)	Pgp (+/+) with DEX
0.5	205.5 ± 113.9	322.4 ± 99.3	18.6	19.5
4	80.9 ± 6.0	82.6 ± 11.3	34.3	47.6
10	10.7 ± 0.4	18.6 ± 6.8	44.0	33.1

[a]Values obtained from pooled sample.

Thus, ondansetron or dexamethasone had no significant inhibitory effect on P-glycoprotein-mediated transport of MK-0869 in CF-1 mice.
Thus, the data suggest that the brain distribution of MK-0869, but not its metabolite L-755444, was dependent on the P-glycoprotein-dependent transport mechanism. The two commonly used antiemetic agents, dexamethasone and ondansetron does not appear to affect the P-glycoprotein-mediated transport of MK-0869 in CF-1 mice.

Investigation of MK-0869 as a Substrate and Inhibitor of P-Glycoprotein.

Methods: MK-0869 was examined as a potential P-glycoprotein (Pgp) substrate and inhibitor in vitro using four cell lines: Caco-2, a human colon adenocarcinoma cell line; KB-VI, a human epidermoid carcinoma cell line with a high level of MDR1 Pgp; L-MDR1, a human MDR1 transfected porcine renal epithelial cell line; and L-mdr 1a, a mouse mdr 1a transfected porcine renal epithelialcell line. Vinblastine, a known Pgp substrate, and cyclosporin A (CsA) and verapamil, two known Pgp inhibitors, were used as positive controls.
Transport experiments in cultured Caco-2 cells were carried out by the addition of 400 µl or 1.5 ml aliqouts of transpoort medium containing [^{3}H]MK-0869 (20 µM) to either the apical (A to B) or basolateral (B to A) compartment, respectively, in the presence or absence of CsA (10 µM) or verapamil (100 µM). The corresponding receiver solutions, without the radiolabeled compound were added in the opposite compartment. After specific time points, aliquots were collected from the receiver compartment and replaced with an equal volume of fresh transport medium. Samples from the donar compartment were collecetd at the end of the 2 hr period. The radioactivity in the samples was measured and the percentage of radioactivity appearing in the opposite compartment was determined. A to B or B to A permeability ratio was calculated and this qualitatively represents the significance of Pgp-mediated transport across the cell monolayer membranes. To examine the potential for MK-0869 as an Pgp inhibitor, similar transport experiments were conducted using [^{3}H]vinblastine as a substrate and unlabeled MK-0869 was used in both the donor and receiver compartments. Similar transport experiments in KB-3-1, KB-VI, MDR1 and mdr 1a cell monolayers were conducted using the method described by Yamazaki et al (J Pharmacol Exp Ther 2001; 296: 723-735).

Results: In Caco-2 cells, MK-0869 was a weak substrate for P-glycoprotein (Pgp), as comapred to vinblastine, a known Pgp substrate. At 20 µM [^{3}H]MK-0869, the permeability (P_{app}) value for B to A transport (BA) was 2.3-fold higher than the permeability for A to B (AB). For vinblastine, the P_{app} value for B to A was about 7-fold higher than that for A to B. The difference between A to B and B to A transport of [^{3}H]MK-0869 was eliminated (BA/AB ratio decreased from 2.3 to 1.07) in the presence of 10 µM CsA (a strong Pgp inhibitor) and was reduced (BA/AB ratio 1.5) in the presence of 100 µM verapamil (a weak inhibitor). The A to B and B to A transport values for MK-0869 and vinblastine in the absence and presence of the Pgp inhibitors, verapamil and CsA are shown in the sponsor's Table below.

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Apparent Permeability Coefficient of [^{3}H]MK-0869 and [^{3}H]Vinblastine from Apical-to-Basolateral (AB) and Basolateral-to-Apical (BA) Directions in Caco-2 Cells With and Without Verapamil or Cyclosporin A (CsA)

Substrate	Inhibitor	P_{app} x10^6 (cm/sec)^a A→B	B→A	(B→A) to (A→B) Ratio
[^{3}H]MK-0869 (20 µM)	Control	2.6 ± 0.6	6.0 ± 0.4	2.3
	+Verapamil (100 µM)	2.5 ± 0.4	3.8 ± 0.1	1.5
	+CsA (10 µM)	4.5 ± 0.2	4.8 ± 1.0	1.07
[^{3}H]Vinblastine (5 µM)	Control	1.8 ± 1.0	12.7 ± 2.4	7.1
	+Verapamil (100 µM)	4.0 ± 0.9	7.2 ± 1.1	1.8
	+CsA (10 µM)	5.2 ± 1.5	5.4 ± 0.6	1.04

^{a}Mean ± SD (n=3).

The accumulation of [^{3}H]MK-0869 was lower in the Pgp overexpressed KB-VI cells (337.8 pmol/well) as compared to that in the parental KB-3-1 cells (536.9 pmol/well; KB-3-1/KB-VI ratio, 1.59). In the presence of CsA, the accumulation of [^{3}H]MK-0869 in KB-VI cells increased to 656.7 pmol/well. Thus, the efflux of [^{3}H]MK-0869 was inhibited by CsA suggesting that MK-0869 may be a substrate for Pgp. The accumulation of [^{3}H]vinblastine to Pgp overexpressed KB-VI cells (18.66 pmol/well) was lower than that in the parental KB-3-1 cells (182.1 pmol/well), and the KB-3-1/KB-VI ratio was 9.76. In the presence of 10 µM CsA, the KB-3-1/KB-VI ratio was much smaller (1.96). In human MDR1 and mouse mdr 1a transfected cell lines, the B to A versus A to B transport ratios for [^{3}H]vinblastine (1 µM) were 11 and 19 respectively. These values were much higher than those observed in Caco-2 and KB cells. The BA/AB ratios for [^{3}H]MK-0869 were also high in both systems. In human MDR1 and mouse mdr 1a transfected cell lines, the BA/AB ratios for [^{3}H]MK-0869 were 7.4 and 13, respectively. In the presence of 10 µM CsA, the ratios were reduced to close to 1. The transport of MK-0869 and vinblastine in human MDR1 and mouse mdr 1a transfected cell lines and its inhibition by CsA is shown in the sponsor's Table below.

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Transport of [³H]MK-0869 and [³H]Vinblastine in Human MDR1 and Mouse mdr1a Transfected Cells Lines

		Transport within 3 hr ($pmol/cm^2$)[a] A→B	B→A	(B→A) to (A→B) Ratio
Human MDR1				
[³H]Vinblastine (1 μM)	Control	5.6 ± 1.7	58.6 ± 4.1	11
[³H]MK-0869 (1 μM)	Control	11.8 ± 1.8	87.5 ± 10.4	7.4
	+ CsA (10 μM)	55.7 ± 5.3	51.4 ± 4.3	0.92
Mouse mdr1a				
[³H]Vinblastine (1 μM)	Control	3.4 ± 1.4	65.9 ± 3.8	19
[³H]MK-0869 (1 μM)	Control	6.7 ± 0.3	84.4 ± 12.9	13
	+ CsA (10 μM)	50.0 ± 1.5	47.3 ± 5.7	0.95

[a]Mean ± SD (n=4).

In Caco-2 cells, MK-0869 (10 μM) had a weak inhibitory activity on the net transport of [³H]vinblastine (1 μM) as compared to CsA and veramapil (both at 10 μM). There were 36%, 87% and 60% inhibitions of Pgp-mediated transport of [³H]vinblastine by MK0869, CsA and verapamil, respectively. Similar results were obtained when a higher concentration (5 μM) of [³H]vinblastine was used as a substrate. In MDR1 overexpressed KV-VI cells, MK-0869 (2 and 10 μM) and verapamil (10 μM) had no substantial effect on [³H]vinblastine accumulation. CsA, on the other hand, caused an increase in the vinblastine accumulation, thus reducing the KB-3-1/KB-VI ratio from 9.76 (control) to 1.96(CsA, 10 μM). The permeability coefficient of [³H]vinblastine in the presence of MK-0869, CsA and verapamil in Caco-2 cells are shown in the sponsor's Table below.

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Apparent Permeability Coefficient of [^{3}H]Vinblastine in the Presence of MK-0869, Cyclosporin A (CsA), and Verapamil in Caco-2 Cells

Substrate	Inhibitor	P_{app} x 10^6 (cm/sec)[a] A→B	B→A	Ratio (B→A)/(A→B)	Inhibition %[b]
[^{3}H]Vinblastine (1 µM)	Control	1.99 ± 0.21	12.6 ± 0.93	6.3	-
	+ CsA (10 µM)	3.86 ± 0.92	6.45 ± 0.49	1.7	87
	+ MK-0869 (10 µM)	2.93 ± 0.47	12.8 ± 1.52	4.4	36
	+ Verapamil (10 µM)	3.52 ± 1.24	11.0 ± 0.93	3.1	60

[a]Mean ± SD (n=3).

[b]% of Inhibition was calculated as: [(Control ratio −1) − (Ratio in the presence of inhibitor −1)]/[(Control ratio −1)] x100%.

Thus, MK-0869 was found to be a substrate of Pgp in Caco-2 and Pgp-overexpressed (KV-VI, MDR1 or mdr 1a transfected cells). However, it was a weaker substrate than the known Pgp substrate, vinblastine.

Brain Penetration of MK-0869 in Male Sprague-Dawley Rats and Male Ferrets.

Methods: The possibility of [^{3}H]MK-0869 to penetrate the blood brain barrier was studied in male Sprague-Dawley rats following i.v and oral administration of a 2 mg/kg dose. The metabolism of [^{3}H]MK-0869 was studied in the plasma and brain collected from the animals. Blood was collected by cardiac puncture at 5, 15 and 30 minutes and 1, 2, 4 and 24 hr after dosing. Brain was collected for determination of brain penetration and isolation of metabolites in brain homogenates. The identity of the MK-0869 metabolites in the plasma and brain were established by comparison with the spectroscopic characteristics of standard authentic metabolites and by LC-MS/MS and NMR analyses. In male ferrets, the possibility of blood brain penetration of MK-0869 and its metabolites was studied after oral administration a 3 mg/kg dose of [^{14}C]MK-0869. Two ferrets were sacrificed at 24 hr after dosing and the remaining 2 ferrets were sacrificed at 48 hr after dosing. Blood and brains were collected at the end of the study to evaluate the brain penetration and isolation of the metabolites.

Results: In the plasma of rats receiving the i.v. dose, 88% of the radioactivity corresponded to the unchanged drug at 5 min post-dose and the plasma concentration of unchanged drug decreased to 1.2% at 24 hr post-dose. In the plasma of rats receiving the oral dose, the unchanged drug represented approximately 74% of the radioactivity at 15 min post-dose, and similar to animals receiving the i.v. dose, the plasma concentration of MK-0869 decreased to 2.5% at 24 hr post-dose. Two metabolites, L-829617 and L-829615, were identified in the plasma of rats receiving the i.v. dose.

Following i.v. dosing, MK-0869 was detected in the brain of rats. The concentration of the parent compound in the brain showed a steady decrease from 116.4 ng/g at 5 min to 46.7 ng/g at 4 hr post-dose (60% decrease). However, the decrease in the concentration of the parent compound was associated with an increase in total radioactivity in the brain (from 185 ng equiv/g at 5 min to 529.7 ng equiv/g at 4 hr post-dose). Rats, receiving an oral dose of 3 mg/kg, had a steadily increase in the brain MK-0869 concentrations from 1.6 ng equiv/g at 15 min post-dose to 21.5 ng/g at 4 hr post-dose. Total MK-0869-related radioactivity increased approximately 46-fold from 15 min (5.7 ng equiv/g) to 4 hr (265.5 ng equiv/g). Three major metabolites were identified in the brain extracts of rats receiving the i.v. or oral doses of MK-0869, and the metabolites were identified as L-825678, L-755446 and L-809861. The plasma and brain total radioactivity and MK-0869 concentrations of rats receiving oral and i.v doses are shown in the sponsor's Table below.

Mean Total Radioactivity (n=3) and Concentrations of [^{3}H]MK-0869 in Plasma and Brain of Sprague-Dawley Rats Dosed Orally or Intravenously With 2 mg/kg [^{3}H]MK-0869

		Plasma		Brain	
Dosing Route	Time (hr)	Total Radioactivity (ng equiv/mL)	MK-0869 (ng/mL)	Total Radioactivity (ng equiv/g tissue)	MK-0869 (ng/g tissue)
P.O.	0.083	2.3±1	BLQ[a]	3.6±0.6	BLQ[b]
	0.25	11±8	8.2	5.7±2.5	1.6
	0.5	54±15	44.6	17.7±0.2	2.4
	1	118±44	77.2	63.6±1.7	6.6
	2	149±27	98.1	103.9±3.5	10.2
	4	281±47	146.1	265.5±1.8	21.5
	24	132±42	3.3	196.2±33.2	BLQ
IV	0.083	785±87	687.7	185.0±2.3	116.4
	0.25	602±33	459.1	219.5±2.2	101.6
	0.5	670±33	457.6	329.4±29.6	89.3
	1	404±241	262.5	275.0±4.6	45.4
	2	567±45	308.3	499.8±20.5	77.0
	4	482±39	181.6	529.7±35.6	46.7
	24	131±9	1.9	151.9±3.5	BLQ

Values represent mean ± SD

[a] The limit of quantification was 1.25 ng/mL.

[b] The limit of quantification was 1 ng/g tissue.

In the brain extracts of ferrets receiving a 3 mg/kg oral dose of [^{14}C]MK-0869, MK-0869 was the major component at both 24 hr and 48 hr post-dose, and represented >60% of the radioactivity. Two minor metabolites, identified as L-825678 and L-755446 were detected in the brain at 48 hr post-dose. In the plasma, [^{14}C]MK-0869 was the major component at both the time points and accounted for 90 to 96% of the plasma radioactivity. In addition three metabolites (L-755446 and L-825678 at 24 hr and L-829617 at 48 hr post-dose) were detected in the plasma of ferrets receiving the oral dose. The total radioactivity and MK-0869 concentrations in the plasma and brain of ferrets receiving an oral dose of [^{14}C]MK-0869 are shown in the sponsor's Table below.

Distribution of [^{14}C]MK-0869 and its Metabolites in Plasma and Brain of Ferrets Dosed Orally With 3 mg/kg [^{14}C]MK-0869

		^{14}C Radioactivity (ng•eq/g tissue)		% ^{14}C Radioactivity as MK-0869[b]	
Ferret #	Time, hr	Plasma[a]	Brain	Plasma	Brain
1	24	610	469	96.1	80
2	24	570	460	96.8	70.5
3	48	393	370	85.4	61.9
4	48	493	332	95.2	68.5

[a] ng•eq/mL plasma.
[b] Estimated from chromatographic profile using HPLC Method A.

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Thus, in contrast to rats, MK-0869 was the major component in both the plasma and the brain of ferrets at 24 hr post-dose.

2.6.4.5 Metabolism

1. **Metabolic conversion of L-758,298 to L-754,030 in subcellular liver fractions from dog and human**

Since L-758,298 is a prodrug for L-754,030, the conversion of L-758,298 to L-754,030 in the liver is necessary for purported *in vivo* biological activity.

Methods: L-758,298 (8.1 μM) was incubated with the liver microsomal or cytosolic fraction at 37° for 0-120 min. After incubation, samples were processed by solid-state extraction and analyzed for L-758,298 and L-754,030 by LC/MS/MS.

Results: As shown in the following table, L-758,298 was converted more rapidly to L-754,030 in human than dog. Furthermore, L-758,298 was converted more rapidly to L-754,030 in microsomes than cytosolic fraction.

Metabolic conversion of L-758,298 to L-754,030 in subcellular liver fractions from dog and human

Enzyme source	Time (min)	L-758,298 (ng/ml)		L-754,030 (ng/ml)	
		Dog	Human	Dog	Human
Microsomes	0	4871	4879	167	313
	15	1424	107	2264	2828
	30	220	ND	2888	2759
	60	25	ND	2886	2745
	120	30	ND	2915	2728
Cytosolic Fraction	0	4584	4617	109	52
	15	3497	4167	1095	496
	30	2518	3720	1826	901
	60	1131	2766	2652	1520
	120	227	1498	3186	2373

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1. Stability of L-758,298 in blood from rat, dog and human

In order to establish assay methods for L-758,298 in plasma, the stability of L-758,298 was studied in blood from rat, dog and human.

Methods: L-758,298 (1 and 10 μg/ml) was incubated in fresh heparinized blood at 37° for 0-120 min. After incubation, vanadate (5 mM) was added immediately and the samples were kept on ice to minimize any *ex vivo* hydrolysis of L-758,298. Blood was centrifuged and 0.2 ml aliquots of plasma were mixed with internal standard, processed by solid-phase extraction and analyzed for L-758,298 and L-754,030 by LC/MS/MS.

Results: As shown in the following table, the stability of L-758,298 was greater in human plasma (< 15% conversion during 2 hrs) than in dog or rat plasma. The stability was greater in dog plasma (30-30 % conversion during 2 hrs) than in rat plasma (half-life of about 30 min).

2. Metabolism of L-754,030 in rat hepatocytes

Methods: Rat hepatocytes were cultured for 48 hr in the presence of 10 μM of dextrose in the culture medium. [4-Fluorophenyl-3-^{3}H]L-754,030 (final concentration of 4.5 μM, 1% DMSO) was added to the hepatocyte culture and incubated at 37° for an additional 24 hr. The cells were harvested and centrifuged, evaporated to dryness and analyzed by HPLC.

In another experiment, [4-fluorophenyl-3-3H]L-754,030 (final concentration of 25 μM, 1% DMSO) was incubated with a freshly prepared rat hepatocyte suspension (15 ml; ~1,000,000 cells/ml) at 37° for 2-4 hr. The reaction was quenched with acetonitrile. The suspension was centrifuged, the supernatant was evaporated to dryness and analyzed by HPLC.

Results: In the case of rat hepatocyte cultures, four metabolites were identified; being the N-dealkylated derivative of L-754,030 that is designated L-755,446, the acetic acid derivative of L-755,446, the acetamide derivative of L-755,446, and the methyl ester of the acetic acid derivative of L-755,446. When the experiment was repeated in the absence of dextrose, an additional unidentified metabolite was found.

In the case of hepatocyte suspensions, three metabolites were identified; being the glucuronide of L-754,030, the acetic acid derivative of L-755,446 and the acetamide derivative of L-755,446.

Mass Balance and Metabolism Studies with [^{14}C]MK-0869 in Dogs.

Methods: The mass balance and biliary excretion of [^{14}C]MK-0869 were studied in beagle dogs following a single 1 mg/kg i.v. or 2 mg/kg oral dose. Blood samples were collected via a jugular vein at 2, 4, 6, 8, 24, 30, 48, 54 and 72 hours after dosing. Urine samples were collected at 0-8, 8-24, and at 24 hours intervals thereafter; feces were collected daily for 7 days. For biliary excretion studies, bile samples were collected from bile duct-cannulated dogs before dosing and at 0-4, 4-8, 8-12, 12-24, 24-48, 48-72, 72-96, and 96-120 hr intervals after dosing. By comparison to authentic metabolite standards and spectroscopic characteristics, the presence of the metabolites in plasma, bile, urine and feces was confirmed by LC-MS/MS.

Results: The excretion patterns of [^{14}C]MK-0869 derived radioactivity were similar in dogs following i.v. (1mg/kg) or oral (2 mg/kg) administration of a single dose. In the dog, fecal and urinary excretions of the radioactivity were similar. Fecal excretions in 168 hours following i.v. and oral administration accounted for 39.1% and 43.1% of the dose and the urinary excretion accounted for 37.7% and 40.5% of the dose, respectively. The average recovery of the radioactivity in excreta in 168 hours, including case rinses were 84.1% and 86.9% following i.v and oral administration, respectively.
The recovery of the radioactivity in the bile of bile duct-cannulated dogs in 5 days following i.v and oral dosing were 49% and 30%, respectively. Several nonpolar (L-755446, L-829674, L-825678, L-809771), polar (L-324261, L-829615, L-829617) and very polar (L-596064, L-770787) metabolites were identified in the plasma of dogs receiving i.v. or oral dosing. MK-0869 was the major radioactive component in the plasma for up to 8 hr post-dose, and L-829617 and L-596064 were predominant at 30 hr postdose. In addition to the nonpolar and polar metabolites identified in the plasma, an additional nonpolar metabolite (L-809861) was identified in the feces; some radioactivity was detected

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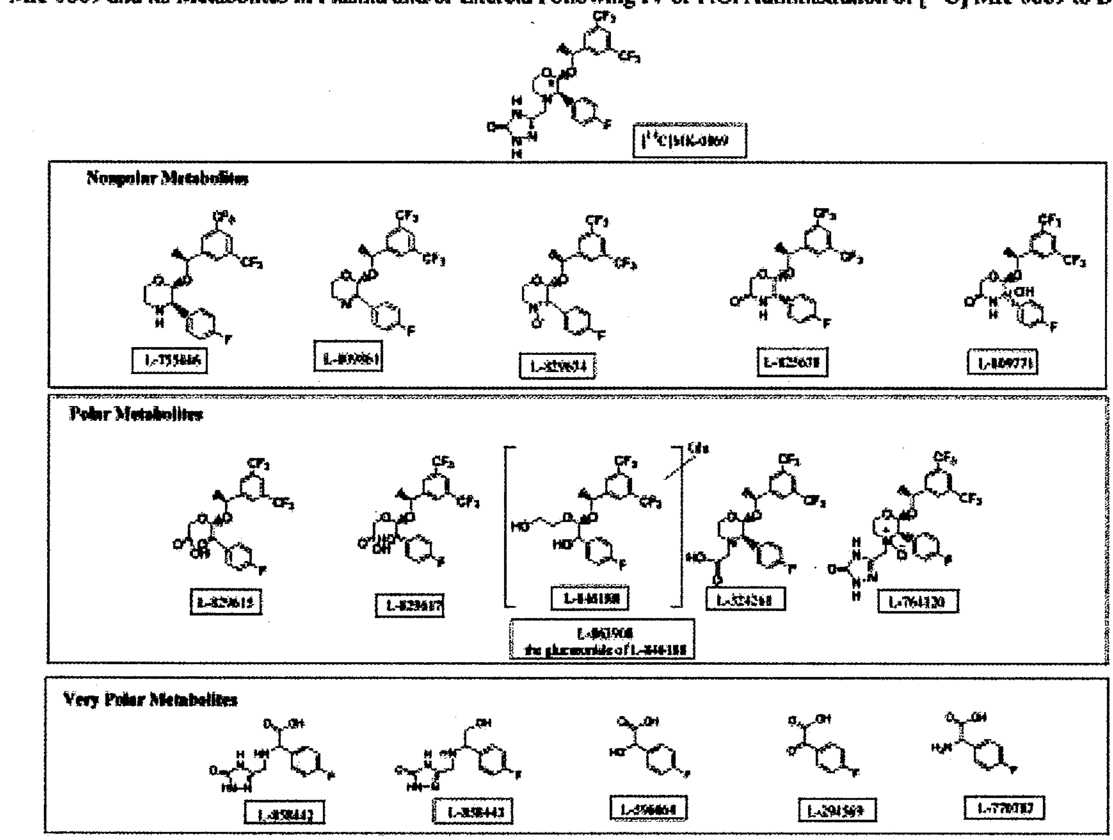
in the very polar region, but not identified. The metabolite profile in the bile was similar to that of feces following both i.v. and oral dosing. In addition, the glucuronide of L-846188, designated as L-863908, was identified in the bile. Four very polar metabolites (L-596064, L-294569, L-858442, L-858443) were identified in the urine of dogs receiving i.v. or oral doses; the major metabolites were L-596064 and L-294569. The structures of the metabolites identified in the plasma and or excreta following i.v. or oral administration in dogs are shown in the sponsor's Figure below.

Figure 1

MK-0869 and its Metabolites in Plasma and/or Excreta Following IV or P.O. Administration of [^{14}C] MK-0869 to Dogs



In Vitro Metabolism of MK-0869 in Microsomes and Primary Cultures of Rat and Human Hepatocytes.

Methods: The metabolism of [morpholine-2-^{14}C]MK-0869 was studied in hepatic microsomes and primary cultures of rat and human hepatocytes. Two primary metabolites of MK-0869, L-755446 (formed by an initial step of N-dealkylation) and L-809861 (an imine metabolite) were used as alternate substrates. For induction of cytochrome P_{450} enzymes in male rats, dexamethasone (200 mg/kg) was administered by oral gavge for 4 days before preparation of microsomes or isolation of the hepatocytes. For studying the oxidative metabolism in liver microsomes, microsomal proteins were incubated with [^{14}C]MK-0869, [^{14}C]L-755446 and [^{14}C]L-809861 for 37°C for 5 to 45 minutes. Primary cultures of rat or human hepatocytes were incubated with these substrates for 4, 6, 24 or 48 hrs (for rat hepatocytes) and 48 hrs (for human hepatocytes). For induction of metabolic enzymes, rat hepatocytes were treated with 10 µM dexamethasone and human hepatocytes were treated with 50 µM rifampicin for 48 hrs prior to the addition of the test compounds. The metabolite profiles of the incubates were determined by LC-MS/MS and nuclear magnetic resonance (NMR) spectroscopy.

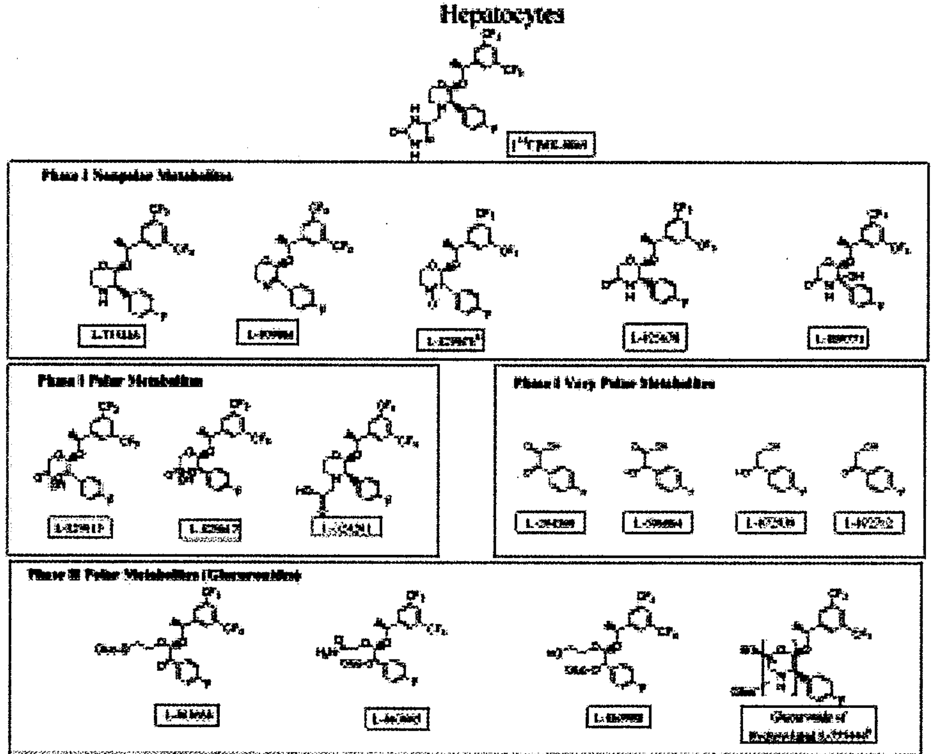
Results:

Metabolism in rat liver microsomes: In liver microsomes prepared from untreated rats, the metabolism of MK-0869 was slow. Two nonpolar metabolites, identified as L-755446 and L-809861 were detected. In addition, three polar (L-829615, L-829617 and L-324261) and four very polar (L-294569, L-596064, L-872939 and L-872712) metabolites were also detected. MK-0869 underwent more extensive metabolism when incubated with liver microsomes prepared from dexamethasone-treated rats.

When L-755446 (the primary metabolite of MK-0869) was used as a substrate, the major metabolite was L-809861. Three very polar metabolites, identified as L-294569, L-872939 and L-872712, were also detected by HPLC. [^{14}C]L-809861 underwent slow metabolism in the presence of rat liver microsomes (<5% conversion in 10 min). Most of the metabolites identified were very polar metabolites.

The structures of the phase1 and phase II metabolites formed after incubation of [14C]MK-0869 with rat and human liver microsomes are shown in the sponsor's Figure below.

Figure 1
The Major Metabolites of [^{14}C]MK-0869[a] Following Incubation With Rat or Human Liver Microsomes Hepatocytes



[a]*Denotes the position of ^{14}C label.
[b]Not found in incubations with human tissues.

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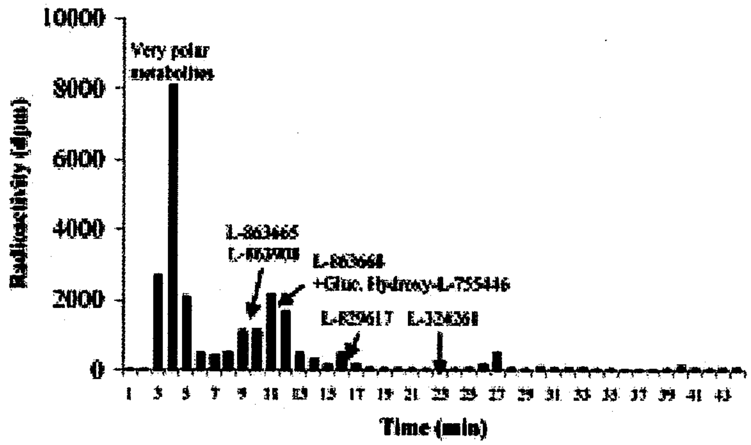
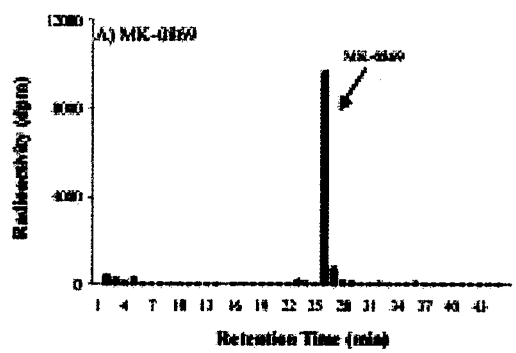
Metabolism in primary cultures of rat hepatocytes: MK-0869 underwent minimal metabolism when incubated with cultured rat hepatocytes. Two of the metabolites had retention times corresponding to those of L-755446 and L-809861. In contrast, [^{14}C]-755446 underwent extensive metabolism in cultured rat hepatocytes and three major metabolites (L-809771, L-755446 and L-825678) were identified. After incubation of [^{14}C]L-809861 with rat hepatocytes for 48 hrs, three metabolites (L-829615, L-829617 and L-809771) were identified by their retention times. There was more extensive metabolism of MK-0869 in the presence of hepatocytes treated with 10 μM dexamethasone.

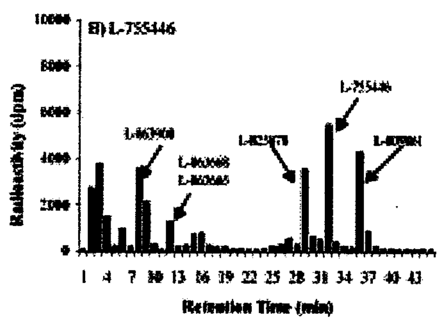
Metabolism in human liver microsomes: The metabolism of MK-0869 by human liver microsomes was slow (<20% in 30 min at 2 mg/ml protein). Three major metabolites (L-324261, L-755446 and L-809861) were identified by HPLC and online LC-MS/MS analysis. The profiles of metabolites of [^{14}C]L-755446 were similar to those from rat microsomes and the major metabolite was identified as L-809861. Incubation of [^{14}C]MK-0869 by rifampicin-treated human hepatocytes was minimal during the 48 hr incubation period. [^{14}C]L-755446 underwent extensive metabolism when incubated with dexamethasone-treated hepatocytes. Three glucuronides, L-863668, L-863665 and L-863908, a lactam (L-825678) and an imine (L-809861) were identified in incubates containing treated hepatocytes. Incubation of [^{14}C]L-755446 with dexamethasone-treated human hepatocytes was associated with the formation of three glucuronides. The representative radioactivity profiles after incubation of [^{14}C]MK-0869 with dexamethasone treated rat and human hepatocytes are shown in the sponsor's Figures below.

Representative Radioactivity Profile of [^{14}C]MK-0869 Metabolism After 72 hr Incubation With Dexamethasone-Treated Rat Hepatocytes



Representative Radioactivity Profiles of [^{14}C]MK-0869 (A) and [^{14}C]L-755446 (B) Metabolism After 48 hr Incubations With Rifampicin-Treated Human Hepatocytes



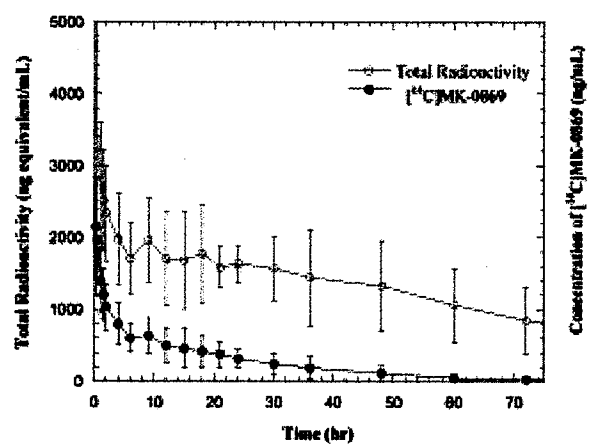


Mass Balance and Metabolism Studies of [^{14}C]L-758298 and [^{14}C]MK-0869 in Humans.

Methods: The pharmacokinetics and metabolism of L-758298 (a water-soluble phosphoramidate prodrug of MK-0869) were studied in healthy male human subjects after i.v. dosing. Each subject received 100 mg (88.8 μCi) of [^{14}C]L-758298, administered by infusion for 30 minutes via an arm vein. For the mass balance study, blood samples were collected at –1, 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60 and 72 hr post-dose and each morning on days 4 to 21. For the metabolism study, blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72 96, 120, 144 and168 hr post-dose. Excreta were collected daily for 30 days.
[^{14}C]MK-0869 was administered orally as capsules containing 100 mg (28.72 μCi) of the active drug in each capsule. Each of the 8 subjects received 3 capsules at a dose level of 300 mg/person. Blood samples were collected at 0.5, 1, 1.5, 2, 4, 6, 9, 12, 15, 18, 21, 24, 30, 36, 48, 60, 72, 96, 120, 144 and 168 hr post-dose. Excreta were collected daily for 10 days. By comparison with authentic metabolite standards and spectropscopic characteristics, the metabolite profiles in the plasma, urine and feces were confirmed by LC-MS/MS and/or NMR analyses.

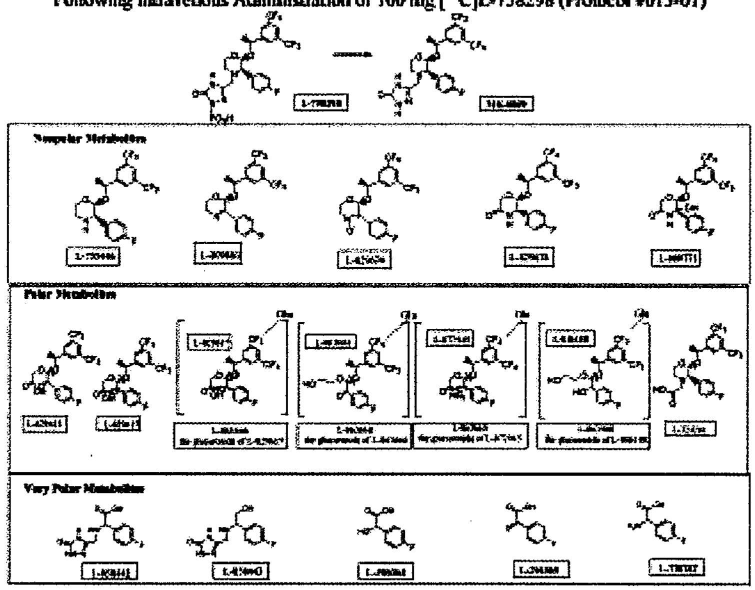
Results:
[^{14}C]L-758298: Following i.v. administration of [^{14}C]L-758298 to healthy human males, about 58% of the radioactivity was recovered in the urine and 45% in the feces during the 28-day collection period. Only 7% of the radioactivity were excreted in the urine collected in days 8-28. In the plasma, L-758298 was rapidly converted to MK-0869. Plasma levels of [^{14}C]MK-0869 were higher at the earliest time point (0.25 hr). At 0.5 hr, [^{14}C]MK-0869 accounted for for 56% of the plasma radioactivity that decliced to 40, 19 and 4% at 4, 24 and 60 hr post-dose, respectively. The mean radioactivity concentrations (total and MK-0869) in the plasma of healthy human subjects after i.v. administration of [^{14}C]L-758298 during the 72-hr period are shown in the sponsor's Figure below.

Mean (±SD) Concentrations of Total Radioactivity and [^{14}C]MK-0869 in Plasma of Eight Healthy Human Subjects Following Intravenous Administration of [^{14}C]L-758298 at 100 mg (Protocol # 013-01)



Several nonpolar (L-755446, L-809861, L-829674, L-825678 and L-809771), polar (L-829615, L-829617 and L-324261) and very polar (L-294569, L-596064 and L-770787) metabolites were identified in the plasma. The two major metabolites in the human plasma were L-829615 and L-829617. L-829674, L-324261 and L-809771 were present at very low concentrations, and L-863664, L-846188 and L-872945 were not detected in human plasma. In feces, several metabolites were identified and those include: L-829617, L-829771, L-825678, L-755446 and L-809771, L-829774 and L-829615. In the urine samples of humans receiving intravenous [^{14}C]L-758298, five very polar metabolites (UM-1 or L-858442, UM-2 or L-858443, UM-3 that had 2 components, L-596064 and L-294569 and UM-4 and UM-5 which consisted of the glucuronides L-86366, L-863665 and L-863908) were detected by HPLC. The major metabolites detected in the plasma, urine and feces of humans following i.v. administration of [^{14}C]L-758298 to healthy human subjects are shown in the sponsor's Figure below.

The Major Metabolites of MK-0869 in Plasma and/or Excreta of Healthy Human Subjects Following Intravenous Administration of 100 mg [^{14}C]L-758298 (Protocol #013-01)



[^{14}C]MK-0869: After oral administration of a 300 mg dose of [^{14}C]MK-0869 to healthy human subjects, about 92% of the radioactivity was recovered in the 10-day collection period, of which 5% was excreted in the urine and 86% excreted in the feces. Intact [^{14}C]MK-0869 accounted for 49% to 64% of the plasma radioactivity during 4 hr following the administration and declined to 30% and 10% by 24 and 60 hr, respectively. By 96 to 168 hr, the plasma levels of the parent drug was below the detection limit. Nonpolar metabolites including L-755446, L-809861, L-829674, L-825678 and L-809771, and polar metabolites including L-829615 and L-829617 were shown to be present in the plasma samples of subjects receiving the oral dose of MK-0869. In the fecal extracts, the major component was the parent compound, MK-0869. In addition, trace levels of the nonpolar and polar metabolites, detected in the plasma, were also detected in the fecal extracts. An additional minor metabolite, identified as L-764120 (an N-oxide of MK-0869), was detected in the feces. Five very polar metabolites (L-596064, L-294569, L-770787, L-858442 and L-858443) and phase II conjugates

L-863665 (glucuronide of L-872945), L-863908 (glucuronide of L-864188) and L-863668 (glucuronide of L-863664) were identified in the urine. Glucuronides of L-755446 (primary nonpolar metabolite of MK-0869) and of hydroxylated L-755446 were also detected in the urine. The structures of the nonpolar, polar and very polar metabolites detected in the plasma, urine and feces of healthy human subjects receiving a 300mg oral dose of [^{14}C]MK-0869 are shown in the sponsor's Figure below.

MK-0869 and its Metabolites in Plasma and/or Excreta of Healthy Human Subjects Following Oral Administration of [^{14}C] MK-0869 at 300 mg (Protocol #013-01)

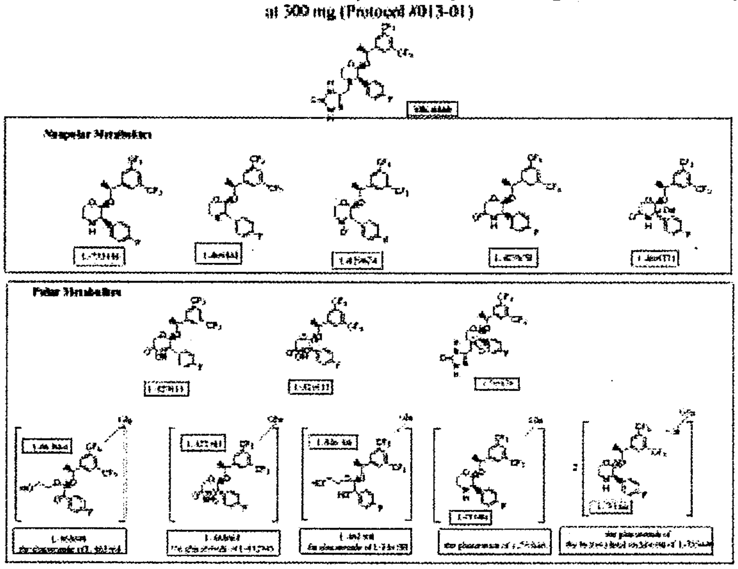
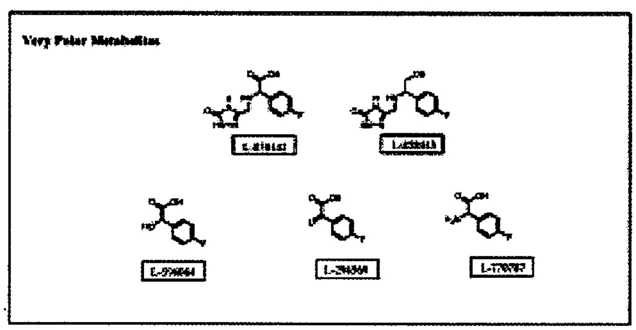


Figure 1 (Cont.)
MK-0869 and its Metabolites in Plasma and/or Excreta of Healthy Human Subjects Following Oral Administration of [^{14}C] MK-0869 at 300 mg (Protocol #013-01)



Thus, the metabolite profiles in the plasma, feces and urine of healthy human subjects were qualitatively similar after i.v administration of the pro-drug, L-758298 and oral administration of MK-0869.