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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-549

Pharmacology Review(s)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

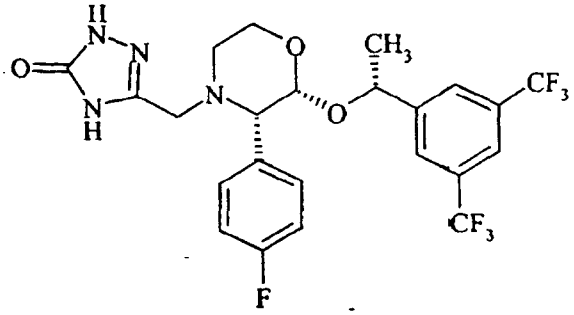
NDA number: 21-549
Review number: 01
Sequence number/date/type of submission: 000/Original/September 30, 2002
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Merck & CO., Inc., West Point, PA 19486
Manufacturer for drug substance: Merck Research Laboratories, West Point, PA.

Reviewer name: Sushanta Chakder, Ph.D.
Division name: Gastrointestinal & Coagulation Drug Productes
HFD #: 180
Review completion date: March 12, 2003

Drug:

Trade name: EMEND
Generic name: Aprepitant
Code name: MK-0869/L-754030
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: 170729-80-3
Molecular formula/molecular weight: $C_{23}H_{21}F_7N_4O_3$/534.43

Structure:



Relevant INDs/NDAs/DMFs: **IND** [redacted], Merck & Co., Inc., West Point, PA.
IND [redacted] 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, including high-dose cisplatin.

Clinical formulation: Each capsule of Emend contains 80 or 125 aprepitant. In addition to the active drug, each capsule contains the following inactive ingredients: sucrose (— —), microcrystalline cellulose (— —), hydroxypropyl cellulose (— — sodium lauryl sulfate NF (— —) and sodium lauryl sulfate NF micronized (— —). The 125 mg capsule also contains red ferric oxide and yellow ferric oxide.

Route of administration: Oral

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

Executive Summary

I. Recommendations

A. **Recommendation on Approvability:** From a preclinical standpoint, the NDA is approvable.

B. **Recommendation for Nonclinical Studies:** None

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

II. Summary of Nonclinical Findings

A. Pharmacologic Activity:

Aprepitant (MK-0869/L-754, 030) is a neurokinin 1 (substance P) receptor antagonist and has been found to bind selectively and with high affinity to NK_1 receptors from different species, and cloned human receptors. It had >3,000-fold higher affnity for NK_1 receptors than NK_3 receptors and had higher affinity than other G-protein coupled receptors. Aprepitant is a brain penetrant as was shown by its ability to cause a dose-dependent inhibition of intracerebroventricular NK 1 receptor agonist-induced foot tapping in gergils. Brain penetration of the compound was also shown in monkeys by positron emission tomography (PET) assay. The antiemetic activity of aprepitant was evaluated in ferrets, and it caused dose-dependent inhibitions of both the acute and delayed phases of retching and vomiting, induced by intravenous cisplatin. In a model of cisplatin-induced delayed emesis, it caused complete inhibition of retching and vomiting at oral doses of 2 and 4 mg/kg. When administered in combination with ondansetron (a 5-HT_3 receptor antagonist), i.v. aprepitant had additive effects on cisplatin-induced emesis in ferrets. Similar additive effects were observed when the drug was administered in combination with dexamethasone. Aprepitant had no effect on the anti-tumor activity of cisplatin in athymic NCr-nu mice bearing a human small cell carcinoma xenograft. In humans, aprepitant in combination with a 5-HT_3 receptor antagonist and dexamethasone, has been found to be effective in preventing both the acute and delayed phases of chemotherapy-induced nausea and vomiting (CINV). L-758, 298 (a prodrug of aprepitant) had no effects on the, respiratory function, blood pressure, heart rate or ECG parameters of anesthetized dogs at an i.v. dose of 1 mg/kg. It had no behavioral and central nervous system (CNS) effects in conscious mice at oral doses up to 100 mg/kg.

Aprepitant is rapidly absorbed after oral dosing in rats, mice and ferrets; the maximum plasma concentration was reached in between 2-4 hours. Saturation of absorption of the drug was observed in rats and mice after oral dosing as evidenced by the plasma concentrations of the parent drug and its metabolites. The oral bioavailability of MK-0869 was 43%, 42.4% and 45.4% in rats, mice and ferrets, respectively. MK-0869 is highly bound to plasma proteins from rats, dogs and humans (>98%). The drug was detected in the fetal plasma, when administered to pregnant animals, and it was excreted in the milk of lactating rats. N-dealkylation is the major pathway of metabolism of L-755, 030. The metabolic profiles of aprepitant were similar in rats, dogs and humans.

B. Toxicological findings:

Acute toxicity studies with L-758, 298 (a prodrug of MK-0869) were conducted in mice and rats after i.v. administration of 200 and 500 mg/kg doses and oral administration of a 500 mg/kg dose. The minimal lethal dose (MLD) by the i.v. route was 500 mg/kg in both mice and rats. There were no deaths of mice or rats receiving the 500 mg/kg oral dose; thus, the MLD by the oral route was not known. The clinical signs observed in mice after i.v. dosing included gasping, convulsions, bradypnea and loss of righting reflex that disappeared within 3 hours. In rats, the clinical signs included gasping and bradypnea.

Subacute/subchronic/chronic toxicity studies wiith MK-0869 were conducted in rats, mice and dogs. In rats, hepatocellular hypertrophy and vacuolation, and thyroid follicular cell heperplasia were observed in both males and females receiving the drug orally for 5 weeks. Benign parafollicular cell adenoma was observed in two treatment group animals (one each at the mid and high doses). In addition to the changes in the liver and thyroid gland, vacuolation of individual cells of pars distalis of the pituitary gland was observed in males receiving MK-0869 for 14-weeks. The liver and the thyroid glands were also the target organs of toxicity in the 27-week and 53-week oral chronic toxicity studies in rats. MK-0869 has been found to increase the microsomal cytochrome P-450 enzyme levels in rats, and the changes observed in the liver and the thyroid gland may be related to the induction of hepatic drug metabolizing enzyme activity. MK-0869 caused an increase in the TSH levels and thyroxine clearance in male and female rats. It caused moderate induction P_{450} enzyme activity in the mouse liver, when administered orally. Elevations of cholesterol and triglyceride levels and hepatocellular hypertrophy were observed in both male and female mice receiving MK-0869 for 5 weeks. Elevetion of cholesterol levels were also observed in rats receiving the drug for 27 weeks. In addition, hydropic degeneration of tubules of kidney was observed in female mice receiving MK-0869 for 14 weeks.

In dogs, testicular degeneration and prostatic atrophy were observed in males receiving 125 mg/kg b.i.d and higher doses p.o. for 5 weeks. An increased incidence of thymic atrophy was also observed in dogs receiving ≥125 mg/kg b.i.d dose. Prostatic atrophy and testicular degeneration were also observed in male dogs receiving 25 mg/kg b.i.d and higher doses for 39 weeks.

The genotoxic potential for L-758, 298 (a pro-drug of MK-0869) was examined by the bacterial reverse mutation assay (Ames assay), the rat hepatocyte DNA damage assay, the mutagenesis assay in TK6 human lymphoblastoid cells and the chromosomal aberrations assay in the Chinese hamster ovary (CHO) cells. It was not found to be genotoxic in any of the assays, either in the absence or presence of metabolic activation. The genotoxic potential for L-754, 030 (MK-0869) was examined by the *in vivo* mouse bone marrow micronucleus assay and the mutagenesis assay in TK6 human lymphoblastoid cells. It was not found to have any genotoxic potential in these assays.

The sponsor conducted two 106-week oral (gavage) carcinogenicity studies in Sprague Dawley rats and a 105-week oral carcinogenicity study in CD-1 mice with MK-0869. In the first carcinogenicity study in rats, oral doses of 0, 0.10, 0.50 and 2.0 mg/kg/day (0, 0.05, 0.25 and 1.0 mg/kg b.i.d.) were used and the exposure levels at the high dose were lower than that in humans at the proposed clinical dose. In this 106-week carcinogenicity study in rats, treatment with MK-0869 was not associated with a significant increase in any type of tumors in the male and female rats. In the second 106-week oral carcinogenicity study in rats, 0, 5, 25 and 125 mg/kg b.i.d (0,

10, 50 and 250 mg/kg/day) doses were used. In this 106-week carcinogenicity study, MK-0869 was carcinogenic to both male and female rats. There were increased incidences of thyroid follicular cell adenoma and carcinoma in the male rats, and hepatocellular adenoma and thyroid follicular cell adenoma in the female rats. The highest tested dose in rats produced a systemic exposure (AUC 0-24 hr) to unchanged drug of 0.4 to 1.4 times the human exposure (plasma AUC 0-24 hr = 19.9 mcg.hr/ml) at the recommended clinical dose. In the 105-week oral carcinogenicity study in mice, 0, 2.5, 25, 125 and 500 mg/kg/day doses were used. MK-0869 was found to be carcinogenic in male and female mice in the 105-week oral carcinogenicity study. Treatment with MK-0869 in mice was associated with increased incidences of fibrosarcoma of the skin in males, and hepatocellular adenoma and harderian gland adenoma in females. The systemic exposure of the parent compound in mice at the highest dose was about 2..2 to 2.7 times the exposure in humans at the recommended dose.

MK-0869 had no treatment-related effects on the fertility and reproductive performance of the male and female rats at doses up to the maximum feasible oral dose of 1000 mg/kg b.i.d (2000 mg/kg/day). MK-0869 was not teratogenic in rats at doses up to the maximum feasible oral dose of 1000 mg/kg b.i.d (2000 mg/kg/day), and in rabbits at oral doses up to 25 mg/kg/day. It had no effects on peri- and post-natal development and reproductive performance of the F_1 generation when administered to pregnat rats from gestation day 6 lactation day 20 at oral doses up to 1000 mg/kg b.i.d. (2000 mg/kg/day).

C. **Nonclinical Safety Issues Relevant to Clinical Use:** None

III. **Administrative**

A. Reviewer signature: /S/

B. Supervisor signature: Concurrence - /S/

Non-Concurrence - _____
(see memo attached)

C. cc: list:

NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Chakder
HFD-180/Dr. Choudary

R/D Init.: J. Choudary 3/5/2003

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PHARMACOLOGY/TOXICOLOGY REVIEW

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Studies reviewed earlier as part of IND [redacted] and IND [redacted] are incorporated here. The new studies submitted are reviewed.

I. PHARMACOLOGY:

Primary Pharmacodynamics:

Receptor Binding:

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

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 LIGAND program [redacted] 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.

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

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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.

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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.

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.

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.

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-HT_{1A}, 5-HT_{2A}, 5-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-HT_{1A}, 5-HT_{2A}, 5-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-HT_{1A}, 5-HT_{2A}, 5-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-HT_{1A}, 5-HT_{2A}, 5-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 [^{3}H]-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.

1c. Receptor selectivity

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 ——— 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:

Aprepitant (MK-0869/L-754, 030) is a Neurokinin$_1$ (Substance P) receptor antagonist. Substance P is present in the sensory nerve terminals and is believed to be involved in nociception. 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 NK_1 receptors. Aprepitant has been shown to bind selectively and with high affnity to NK_1 receptors from rat, dog, guinea pig, ferret and human. It has also been shown to inhibit the binding of [^{125}I]-L-703, 606 (a selective NK_1 receptor antagonist) to human NK_1 receptors, and inhibit the substance P-induced inositol tris-phosphate (IP_3) synthesis in Chinese Hamster ovary cells, expressing human NK_1 receptors. Thus, the anti-emetic effect of aprepitant might be due to antagonism of NK_1 receptors at the central level.

Drug activity related to proposed indication:

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

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

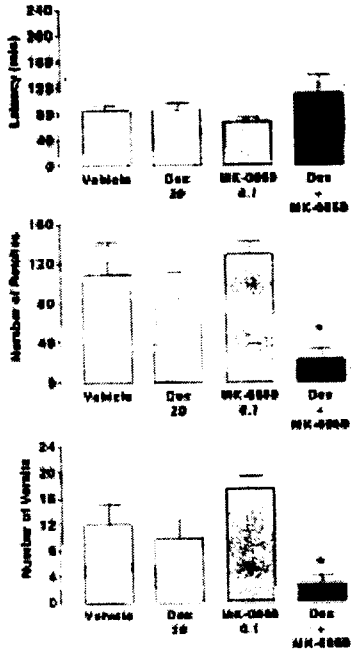
Inhibitory Effects of Combined Treatment with MK-0869 and the Steroid Dexamethasone on Cisplatin-Induced Emesis in Ferrets.

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).

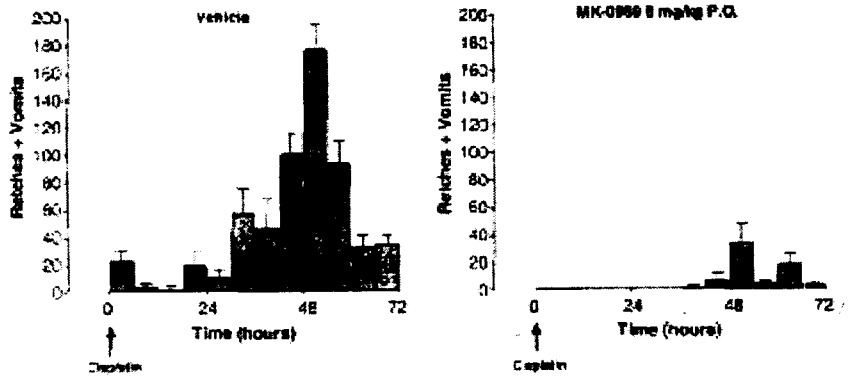
Inhibition of Cisplatin-Induced Delayed Emesis in Ferrets by MK-0869.

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).
(IP) Notebook/Page: 11899/291 and 292; 9863/73
Study Period: Oct - Nov 1996

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ON ORIGINAL

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.

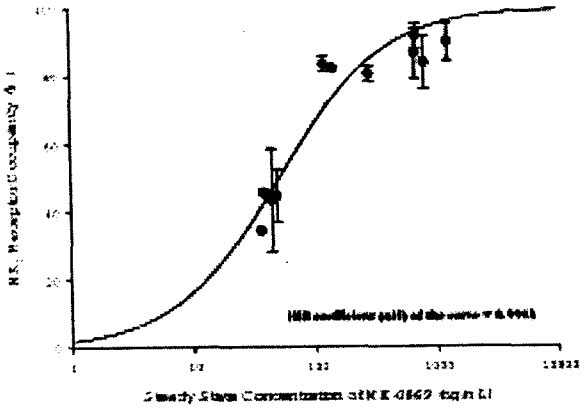
Inhibition of morphine-induced (0.5 mg/kg, s.c.) emesis by orally administered L-754,030 and L-758,298 in ferrets

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.



Note:

Secondary Pharmacodynamics:

Inhibition of centrally-medicated NK_1 agonist-induced foot tapping in gerbils

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).

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.

Inhibition of substance P-induced dermal inflammation in the guinea pig

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 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 Ca-entry. The highest concentration of L-254,030 (10 μM) had a weak effect on Ca-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 model, the present study assessed whether either L-758,298 or L-254,030 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° C. 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-758,030; 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 5-HT. In contrast, L-758,298 and L-758,030 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 [redacted]

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:

Aprepitant (MK-0869/L-754, 030) is a neurokinin$_1$ (substance P) receptor antagonist and has been found to bind 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 and guinea pig NK_1 receptors, and to NK_1 receptors from dog and ferret cerebral cortex, with IC_{50} values in the nM range. It has >3,000-fold higher affnity for NK_1 receptors than NK_3 receptors and has 818-fold higher affinity than other G-protein coupled receptors (α_{1a}, α_{1b}, α_{1c}, α_{2a}, α_{2b}, α_{2c}, β_1, β_2, β_3; muscarinic M_1, M_2, M_3, M_4 and M_5; dopeminergic D_2, D_3 and D_4). The specificity for binding of aprepitant to NK_1 receptors 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_1 receptors. It has been found to inhibit substance P-induced IP_3 formation in Chinese Hamster Ovary (CHO) cells expressing human NK_1 receptors. After i.v. dosing of L-758, 298 (a phosphorylated pro-drug) to rhesus monkeys, a good relationship between plasma active drug (MK-0869) concentration and central receptor occupancy (determined by PET assay) was observed. Intravenously administered aprepitant (1.0 mg/kg) caused inhibition of facilitation of the nociceptive spinal flexor reflex in a rabbit model. Several *in vivo* studies have been conducted to examine the anti-emetic effects of aprepitant, either alone, or in combination with other agents. Oral or i.v doses of aprepitant caused dose-dependent increase in the latency of cisplatin-induced retching and vomiting and reduced numbers of retches and vomits. At an oral dose of 3 mg/kg and an i.v dose of 1 mg/kg, it caused complete inhibition of cisplatin-induced retches and vomits. In a model of cisplatin-induced delayed emesis in ferrets, MK-0869 caused complete inhibition of retching and vomiting at oral doses of 2 and 4 mg/kg. When administered in combination with ondansetron (a 5-HT_3 receptor antagonist), i.v. aprepitant had additive effects on cisplatin-induced emesis in ferrets. Similar additive effects were observed when the drug was administered in combination with dexamethasone. At suboptimal doses, dexamethasone (20 mg/kg i.v) and MK-0869 (0.1 mg/kg i.v) were not effective in preventing cisplatin-induced emesis in ferrets, but the combination caused significant inhibition of emesis in this animal model.

Pharmacology conclusions:

Substance P, a tachykinin, is present in the sensory nerve terminals, and is most likely involved in nociception. Substance P may have important role in emesis, as substance P-containing vagal afferent fibers innervate the brainstem nucleus tactus solitarius, a region in the CNS involved in emesis. Aprepitant (MK-0869 or L-754, 030) is a selective and highly potent NK_1 (substance P)

receptor antagonist, as shown by its affinities for native and cloned receptors from diffreent species. It has been found to cause an inhibition of facilitation of the nociceptive spinal flexor reflex in a rabbit model. The antiemetic effect of aprepitant has been assessed, either alone or in combination, in different preclinical 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. The preclinical pharmacology studies indicate that aprepitant might be effective in preventing chemotehrapy-induced emesis in cancer patients.

II. SAFETY PHARMACOLOGY:

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% methyl-cellulose) 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.

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.

Inhibition of Separation-Induced Vocalizations in Guinea Pig Pups by MK-0869.

The effects of three doses of MK-0869 on separation-induced vocalizations in guinea pig pups were examined after oral administration of 0.3, 1 and 3 mg/kg doses of the drug.

Pups isolated from their mother and litter-mates had vigorous vocalization response during the 15 minutes separation period. MK-0869 caused almost complete inhibition of the vocalization response at 3 mg/kg with an ID_{50} of 0.73 mg/kg.

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.

Safety pharmacology summary:

The safety pharmacology studies were conducted with phosphorylated pro-drug of L-754, 030, L-758, 298. 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 mg/kg. Substance P-induced depressor response and tachycardia in anesthetized dogs were reversed by L-758, 298. It caused an increase in the urine flow and sodium excretion in dogs at an oral dose of 5 mg/kg; however, no other renal effects were observed in dogs. L-758, 298 had no behavioral and 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) and hemostasis (mean arterial pressure, heart rate, systolic and diastolic blood pressures) of anesthetized dogs. It had no effects on the gastric acid output of conscious dogs and on gastrointestinal motility of conscious mice.

Safety pharmacology conclusions:

The safety pharmacology studies conducted with L-758, 298 (the pro-drug) in different animal models. Only treatment-related effects observed were increased urine output and sodium excretion in dogs. Thus, the safety pharmacology studies do not raise any safety alarm for the compound. However, none of the safety pharmacolgy studies were conducted with the active drug, L-745, 034.

III. PHARMACOKINETICS/TOXICOKINETICS:

1. 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 [redacted] 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

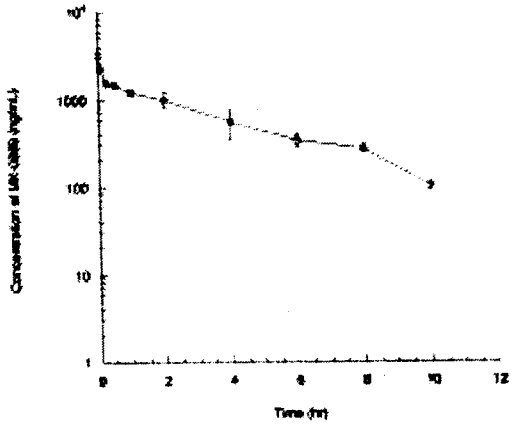
Parameter	L-758,298 (mg/kg, i.v.)		
	1	8	25
Dose (μmol/kg)	1.00	7.96	24.88
AUC of L-754,030 (ng•hr/ml)	568	6294	22392
Average AUC/μmol dose (ng•hr/ml)	568	785	900
Parameter	L-754,030 (mg/kg, i.v.)		
	0.2	2	5
Dose (μmol/kg)	0.37	3.74	9.36
AUC of L-754,030 (ng•hr/ml)	201	2658	6377
Average AUC/μmol dose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

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

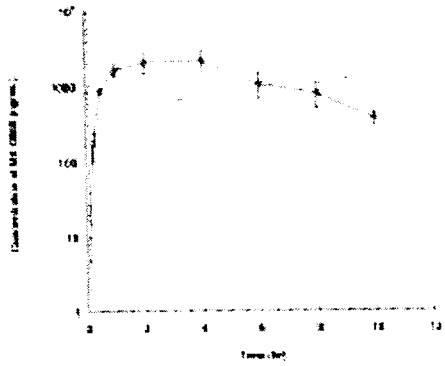
Methods: The pharmacokinetics and oral bioavailability of MK-0869 were determined after i.v (2 mg/kg) and oral (10 mg/kg) administration to male CD-1 mice. For i.v. administraion, the drug was dissolved in ethanol/propylene glycol/water and for oral administration, it was suspended 0.5% methylcellulose 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 [redacted] 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



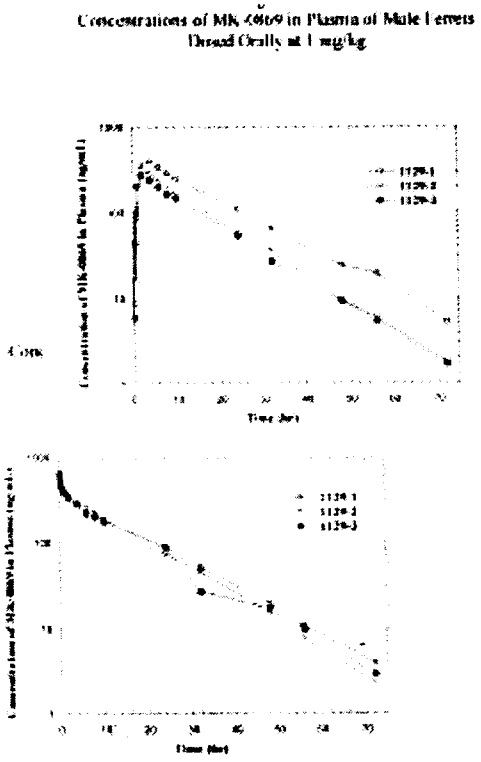
Concentrations (Mean ± SD, n=3) of MK-0869 in Plasma of Male CD-1 Mice
Dosed Orally at 10 mg/kg



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 [redacted] 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.



1. Metabolism of [4-Flurophenyl-3-^{3}H]L-754,030] after intravenous administration

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

Methods: Rats were prepared with cannulas in the common bile duct for bile collection. [4-Flurophenyl-3-^{3}H]L-754,030] was prepared in ethanol:propylene glycol:water [13:58:2,(v/v/v)] at a specific activity of 33.6 μCi/mg. Rats were intravenously administered 2 mg/kg of [4-Flurophenyl-3-^{3}H]L-754,030] via the tail vein. Plasma samples were obtained from 2 rats/sampling time by cardiac puncture at 0.5 and 2 hr after dosing. After dosing, rats were housed in metabolism cages, and bile, urine and feces were collected for 3 days. Samples from plasma, bile, urine and feces were prepared and subjected to — analysis.

Results: When plasma samples were obtained at 0.5 hr after dosing and subjected to — analysis, the parent compound accounted for all the radioactivity. When plasma samples were obtained at 2 hr after dosing and subjected to — analysis, the parent compound accounted for 91% of the radioactivity; metabolites RP-1 and RP-2 accounted for 5% and 4% of the radioactivity.

When urine samples (0-24 hr) were subjected to — analysis, radioactivity eluted as a single peak that probably represented a mixture of many components.

When feces (0-24 hr) were subjected to — analysis, peaks identified as RF-1, RF-2 and parent compound accounted for 8%, 5% and 16% of the dose, respectively; in the case of 24-48 hr samples, 5%, 3% and 2% of the dose, respectively.

When bile samples (0-48 hr) were subjected to — analysis, RB-1 and parent compound accounted for 9% and 7% of dose, respectively; other metabolites for 0.4-3% of the dose. When bile samples were treated with β-glucuronidase and subjected to — analysis, the RB-1 peak decreased and the parent compound increased, suggesting that RB-1 is the glucuronide of L-754,030. — analysis confirmed that RB-1 is the glucuronide of L-754,030. Finally, a glucuronide of L-754,030 (L-777,141) was synthesized and compared with RB-1. Characteristics of RB-1 and L-777,141 were similar.

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 —

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 —

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.

1. Excretion of [4-flurophenyl-3-^{3}H]L-754,030] after intravenous administration

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

Methods: [4-Flurophenyl-3-^{3}H]L-754,030] was prepared in ethanol:propylene glycol:water [13:58:2,(v/v/v)] at a specific activity of 33.6 μCi/mg. Three rats were intravenously administered 2 mg/kg of [4-Flurophenyl-3-^{3}H]L-754,030] via the

tail vein. After dosing, rats were housed in metabolism cages, and urine and feces were collected for 4 days. Radioactivity was determined by scintillation counters.

Results: As shown in the following table, 41% of the total dose of intravenously administered [4-flurophenyl-3-^{3}H]L-754,030] was recovered from the urine over 96 hr after dosing, and 58% in the feces. These data suggest the possibility that fecal excretion, presumably via bile, is a major pathway for the elimination of L-754,030 in rats.

Percent of radioactive [4-flurophenyl-3-^{3}H]L-754,030 (2 mg/kg, i.v.) recovered from urine and feces

Time (hr)	Percent of radioactive dose	
	Urine	Feces
0-24	29.9	38.9
24-48	7.6	13.9
48-72	2.2	3.6
72-96	1.1	1.5
Total	**41**	**58**

2. Excretion of [4-flurophenyl-3-^{3}H]L-754,030] after intravenous administration in bile duct-cannulated rats

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

Methods: Rats were prepared with cannulas in the common bile duct for bile collection. [4-Flurophenyl-3-^{3}H]L-754,030] was prepared in ethanol:propylene glycol:water [13:58:2,(v/v/v)] at a specific activity of 33.6 μCi/mg. Three rats were intravenously administered 2 mg/kg of [4-Flurophenyl-3-^{3}H]L-754,030] via the tail vein. After dosing rats were housed in metabolism cages, and bile, urine and feces were collected for 3 days. Radioactivity was determined by scintillation counters.

Results: As shown in the following table, 51% of the total dose of intravenously administered [4-flurophenyl-3-^{3}H]L-754,030] was recovered from the bile over 72 hr after dosing; 23% in the urine and 8% in feces. These data confirm that biliary excretion is a major pathway for the elimination of L-754,030 in rats.

Percent of radioactive [4-flurophenyl-3-^{3}H]L-754,030 (2 mg/kg, i.v.) recovered from bile, urine and feces

Time (hr)	Percent of Radioactive dose		
	Bile	Urine	Feces
0-8	17.7		
8-24	19.8		
0-24		14.4	2.7
24-48	9.7	5.8	1.4
48-72	3.7	2.4	0.2
Total	51	23	4

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 _ —— 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.

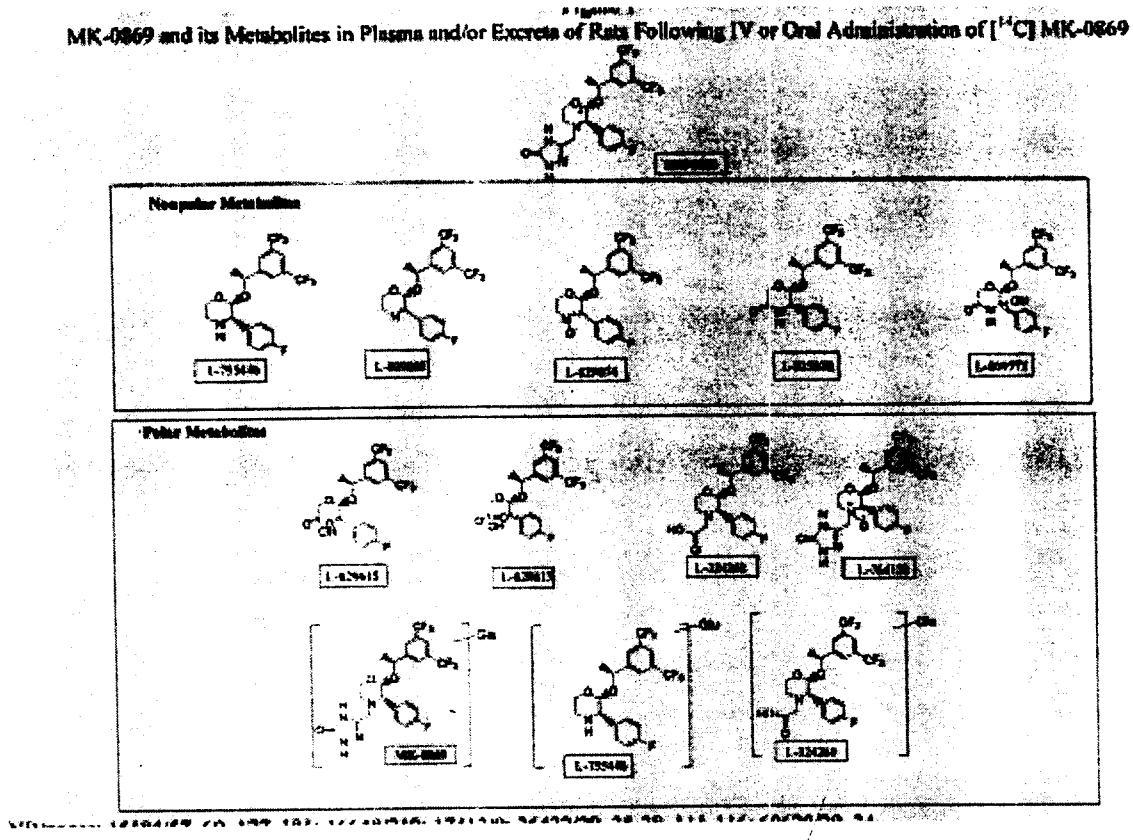
Mean (n=3) Tissue Concentrations of Radioactivity in Rats Following IV Administration of [^{14}C]MK-0869 at 2 mg/kg

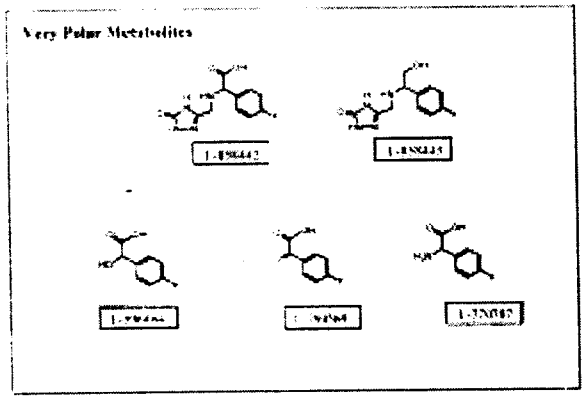
Tissue	MK-0869 Radioconcentration (µg/g)							
	5 Min		30 Min		4 Hours		24 Hours	
	Mean	SD*	Mean	SD	Mean	SD	Mean	SD
Adrenal glands	15.8	4.65	7.48	0.642	4.03	0.170	0.617	0.309
Blood	0.807	0.101	0.503	0.026	0.422	0.011	0.096	0.003
Bone (both femurs)	0.857	0.047	0.884	0.044	0.658	0.084	0.115	0.014
Bone 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#	NA#	NS	NA	0.261	0.036
Eyes	0.443	0.121	0.355	0.038	0.265	0.016	0.032	0.026
Fat (epididymal area)	0.296	0.015	0.910	0.021	2.19	0.268	1.59	0.071
Heart	1.16	2.71	1.79	0.689	2.28	0.211	0.421	0.121
Kidneys	7.37	0.575	5.07	0.254	3.10	0.308	0.618	0.123
Large intestine	0.853	0.047	1.43	0.166	1.54	0.160	0.415	0.012
Large intestinal contents and wash	0.010	0.000	0.051	0.018	0.188	0.001	0.474	0.114
Liver	13.0	1.81	11.4	0.162	9.15	0.521	1.55	0.225
Lungs	12.0	2.56	4.18	0.356	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	0.094
Muscle (thigh)	1.62	0.358	1.77	0.240	1.22	0.086	0.160	0.015
Pancreas	4.12	0.151	5.05	0.535	2.98	0.230	0.479	0.035
Pituitary	1.09	1.94	1.09	1.65	2.29	0.361	0.304	0.162
Placenta	1.38	0.148	0.768	0.183	0.697	0.090	0.120	0.006
Prostate	0.651	0.100	1.22	0.207	1.88	0.350	0.351	0.033
Skin (dorsal, thoracic)	0.348	0.060	0.679	0.101	0.871	0.130	0.185	0.012
Small intestine	5.50	0.126	7.41	0.211	5.72	0.591	0.460	0.035
Small intestinal contents and wash	0.168	0.046	3.18	0.478	3.07	0.984	0.414	0.065
Spleen	2.39	0.362	2.40	0.081	1.50	0.080	0.346	0.040
Stomach	2.20	0.117	2.39	0.121	1.64	0.178	0.201	0.021
Stomach contents and wash	0.084	0.000	0.071	0.080	0.127	0.030	0.016	0.046
Testes	0.073	0.005	0.122	0.015	0.430	0.012	0.256	0.022
Thymus	1.34	0.148	1.34	0.035	1.50	0.094	0.262	0.037
Thyroid/parathyroid	9.26	2.29	3.65	0.252	2.59	0.228	0.518	0.086
Urinary bladder	0.504	0.038	0.790	0.038	1.64	0.572	0.679	0.072

*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.





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 ——— the major metabolites in urine were L-596064 and L-294569.

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 ——— 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%

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

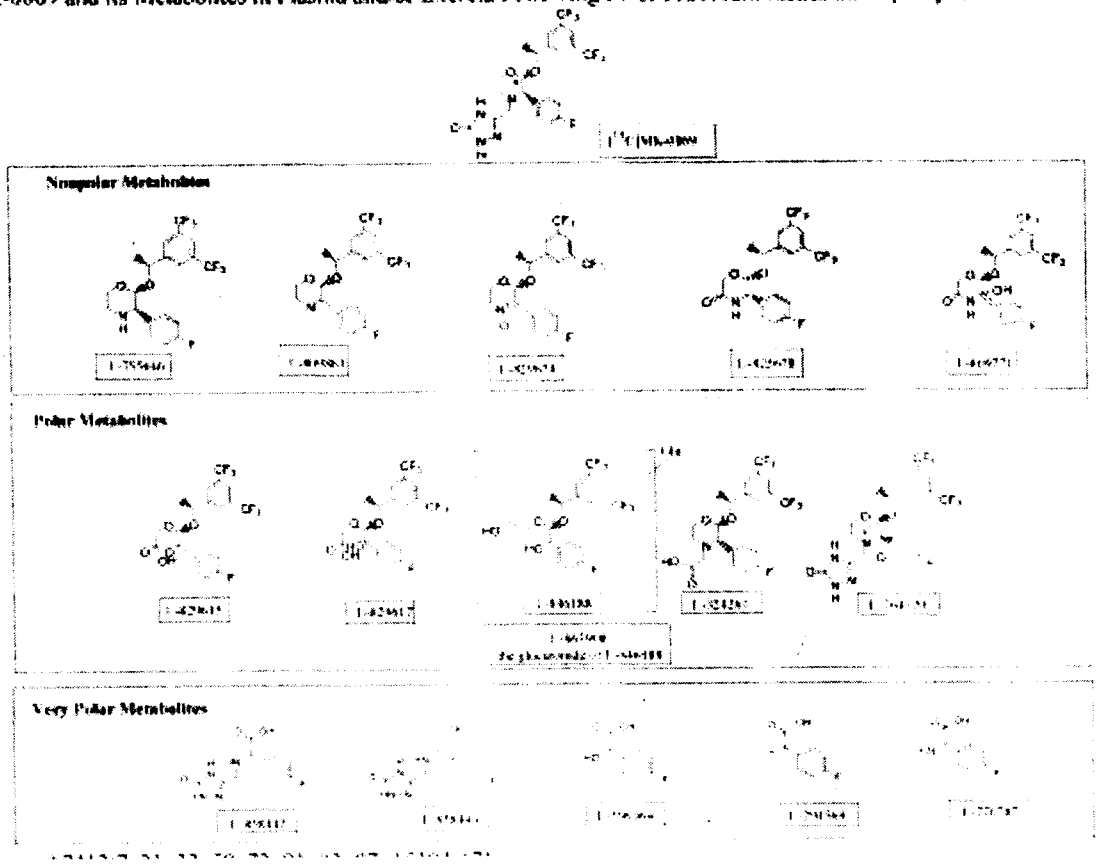
Methods: The mass balance and biliary excretion of [^{14}C]MK-0869 were studied in beagle dogs following a single 1 mg/kg i.v. or 2 mg/kg oral dose. Blood samples were collected via a jugular vein at 2, 4, 6, 8, 24, 30, 48, 54 and 72 hours after dosing. Urine samples were collected at 0-8, 8-24, and 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 characteristics, the presence of the metabolites in plasma, bile, urine and feces was confirmed by

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

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



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 ——— and analyzed for L-758,298 and L-754,030 by ———

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 and analyzed for L-758,298 and L-754,030 by

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. 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.

Conversion of L-758,298 to L-754,030 in plasma from rat, dog and human

L-758,298 added to blood (μg/ml)	Time (min)	Concentration of L-758,298 in plasma (μg/ml)			Concentration of L-754,030 in plasma (μg/ml)		
		Rat	Dog	Human	Rat	Dog	Human
1	0	1.45	1.65	1.64	0.05	0.10	0.17
	15	1.08	1.56	1.62	0.29	0.15	0.17
	30	0.68	1.55	1.58	0.47	0.20	0.19
	60	0.37	1.38	1.56	0.65	0.29	0.28
	120	0.12	1.16	1.52	1.00	0.40	0.45
10	0	14.30	15.90	15.80	0.34	0.64	0.79
	15	10.90	15.80	16.40	3.10	0.96	0.97
	30	8.16	15.00	15.80	4.29	1.28	1.10
	60	3.95	14.40	16.20	6.14	2.07	1.40
	120	1.16	12.50	15.80	8.16	3.23	2.32

2. Distribution:

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.