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APPLICATION NUMBER:
21-337

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA
Division of Anti-infective Drug Products, HFD-520

NDA: 21-337 (000)

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KEY WORDS: MK-0826, Ertapenem, Invanz

Date of submission: November 30, 2000

Review completion date: May 3, 2001

Number of volumes: 31

Relevant INDs: _____

Information to sponsor: Yes (x) No ()

Sponsor: Merck Research Laboratories
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Drug:

Code names: MK-0826, L-749345, ICI-251595, ZD-4433

Generic name: Ertapenem sodium

Trade name: Invanz

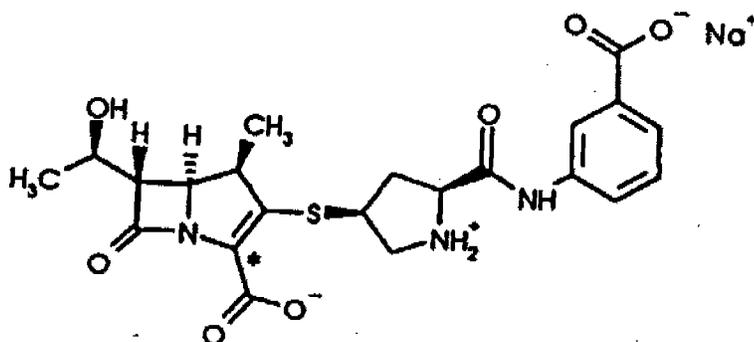
Chemical Name: [4R-[3(3S*,5S*),4 α ,5 β ,6 β (R*)]]-3-[[5-[[[(3-carboxyphenyl)amino]carbonyl]-3-pyrrolidinyl]thio]-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monosodium salt

Chemical Abstracts Services Registry Number: 153832-38-3

Molecular Formula: C₂₂H₂₄NaN₃O₇S

Molecular Weight: 497.5

Structure (showing position of radioactive label for PK studies)



*Position of ¹⁴C-label.

TABLE OF CONTENTS

INTRODUCTION	
SAFETY PHARMACOLOGY	3
PHARMACOKINETICS	3
Absorption	4
Distribution	5
Metabolism	6
Excretion	7
Protein Binding	8
Maternal/Fetal Levels	9
TOXICOLOGY	9
Single-dose Toxicology Studies	10
Repeat-dose Toxicology Studies	11
Studies in Rats	12
Studies in Rabbits	12
Studies in Monkeys	20
Reproductive Toxicology Studies	21
Genetic Toxicology Studies	31
Special Toxicology/Tolerance Studies	34
OVERALL SUMMARY AND EVALUATION	36
RECOMMENDATIONS	38
	39

INTRODUCTION

L-749,345 (MK-0826) is a carbapenem (beta-lactam) antibiotic that is intended for intravenous and intramuscular use to treat a wide range of bacterial infections.

MK-0826 is an optically active, hygroscopic molecule that contains six chiral centers. It has an optical rotation of plus 230 degrees. The compound is soluble in water (>500 mg/ml) and has an ultraviolet absorbance maximum at 294 nanometers.

MK-0826 will be available as a bicarbonate-buffered, lyophilized formulation containing one gram of the free acid per 20 ml vial. The intended clinical dose is one gram once daily. MK-0826 is extensively bound to plasma proteins, and has an elimination half-life in humans of approximately four hours.

SAFETY PHARMACOLOGY

L-749345 was screened for convulsant activity in the rat intracisternal model, and the following results were reported in brief memo format:

Compound	Dose (micrograms/rat)	Convulsant Response
Saline (negative control)	0 (0.01 ml/rat)	0/4
L-749345	12.5	0/12
L-749345	25	0/8
L-749345	50	1/12
L-749345	100	3/8
L-749345	200	9/12
L-749345	400	7/8
Imipenem (positive control)	20	5/8

PHARMACOKINETICS

MK-0826 concentrations in plasma, bile, and urine were measured using (in early studies) a validated bioassay, and later, a validated, ~~method~~ method. Both methods had a quantification limit of ~~—~~ mcg/ml. In studies involving radioactivity, the molecule was labeled with ¹⁴C, and radioactivity was determined by liquid scintillation spectrometry. Note that AUCs were expressed in units of either mcg/ml x minutes or mcg/ml x hours. Half-lives were expressed as means or harmonic means.

The following pharmacokinetic parameters were reported for rats and monkeys after single intravenous doses ranging from 10-180 mg/kg. Note the substantial differences in half-life, clearance, and AUC (0-infinity) between rodents and primates. Also note the differences between unbound and total plasma concentrations.

Pharmacokinetics of MK-0826 Based on Total Plasma Concentrations in Rats and Monkeys After IV Administration (Mean ± SD, n=3-4)

Species	Dose (mg/kg)	AUC (µg/mL x min)	Clearance (mL/min/kg)	Half-life (min)
Rat	10	2033 ± 504	5.17 ± 1.34	27 ± 10
	30	3202 ± 303	9.42 ± 0.83	35 ± 8
	60	5836 ± 719	10.4 ± 1.3	36 ± 5
	180	6862 ± 1718	27.5 ± 6.9	50 ± 9
Monkey	10	34577 ± 6956	0.30 ± 0.06	302 ± 24
	30	44258 ± 9062	0.70 ± 0.14	290 ± 25
	60	76542 ± 10877	0.80 ± 0.10	319 ± 45
	180	106885 ± 9510	1.70 ± 0.15	282 ± 12

Pharmacokinetics of MK-0826 Based on Unbound Plasma Concentrations in Rats and Monkeys After IV Administration (Mean ± SD, n=3-4)

Species	Dose (mg/kg)	AUC (µg/mL x min)	Clearance (mL/min/kg)
Rat	10	96 ± 24	110 ± 30
	30	223 ± 21	136 ± 12
	60	510 ± 63	119 ± 14
	180	1206 ± 347	159 ± 46
Monkey	10	556 ± 122	18.7 ± 4.3
	30	917 ± 209	33.9 ± 7.2
	60	2431 ± 557	25.6 ± 5.1
	180	7603 ± 1047	24.0 ± 2.9

The following pharmacokinetic parameters were reported for mouse, rat, monkey, chimpanzee, and human following a single intravenous dose of either 10 or 14 mg/kg:

Species Comparison of Pharmacokinetics of MK-0826 After IV Administration (Mean \pm SD)

Species	Dose (mg/kg)	α_p (mL/min/kg)	$v_{d_{ss}}$ (mL/kg)	$t_{1/2}$ (min)
DBA/2 Mouse ^a	10	5.81	135	16
Rat (n=4)	10	5.17 \pm 1.34	145 \pm 31	27 \pm 10
Monkey (n=4)	10	0.30 \pm 0.06	84 \pm 8	302 \pm 24
Chimpanzee (n=1)	10	0.37	126	264
Human (n=16)	14 ^b	0.44 \pm 0.08	119 \pm 12	228 \pm 32 ^c

^aMean plasma concentrations from three DBA/2 mice at each sampling time were used in the estimation of pharmacokinetic parameters.

^bThe mg/kg dose was calculated based on a mean body weight of 70 kg.

^cHarmonic mean for $t_{1/2}$ with jackknife standard deviation.

Absorption

~~MK-0826 was absorbed after subcutaneous administration, but was poorly absorbed after oral administration.~~ In a mouse septicemia model, the ED50 was 80 times higher after oral administration than after subcutaneous administration, suggesting an oral bioavailability of approximately 1.25% in mice.

A study was conducted in six (cilastin-pretreated) rats to compare the AUCs achieved following oral and subcutaneous dosing. Three rats received an oral dose of 10 mg/kg, while three other rats received the same dose subcutaneously. The AUCs after oral and subcutaneous doses were 13.43 and 214.1 respectively, suggesting an oral bioavailability of approximately 6.3% in rats.

Because of the poor oral absorption, the compound is not suitable for oral administration.

A study was conducted in six rhesus monkeys to compare the AUCs achieved following intravenous and intramuscular doses of 10 mg/kg. The mean AUC in the three animals treated by the intramuscular route was slightly higher than the mean AUC in the three animals that had been dosed intravenously, suggesting an intramuscular bioavailability of 100%.

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Distribution

The distribution pattern shown in the following table was seen following the intravenous administration of a radioactive dose (15 mg/kg) to rats. The highest levels were found in the kidneys, plasma, small intestine, skin, liver and adrenals.

Tissue Concentrations of Radioactivity in Rats After IV Administration of 15 mg/kg [¹⁴C]MK-0826

Tissue	MK-0826 Radioequivalents ($\mu\text{g/g}$ tissue) ^a			
	0.5 Hr	3 Hr	24 Hr	72 Hr
Heart	4.99 \pm 1.66	0.53 \pm 0.30	0.18 \pm 0.02	0.07 \pm 0.01
Lungs	5.80 \pm 2.31	1.19 \pm 0.09	0.21 \pm 0.05	0.15 \pm 0.01
Liver	18.2 \pm 3.7	55.4 \pm 12.7	11.9 \pm 1.8	0.36 \pm 0.05
Kidneys	71.7 \pm 25.0	81.9 \pm 25.6	7.82 \pm 0.39	5.55 \pm 0.74
Spleen	3.31 \pm 1.04	2.10 \pm 2.47	0.16 \pm 0.05	0.65 \pm 0.15
Testes	4.54 \pm 0.60	1.04 \pm 0.41	0.10 \pm 0.01	0.08 \pm 0.01
Stomach	4.72 \pm 1.10	0.68 \pm 0.18	1.59 \pm 0.92	0.08 \pm 0.01
Small Intestine	31.5 \pm 7.3	22.1 \pm 13.3	1.99 \pm 0.92	0.08 \pm 0.01
Large Intestine	4.16 \pm 3.60	0.80 \pm 0.38	7.58 \pm 2.15	0.12 \pm 0.04
Cecum	6.31 \pm 0.73	2.61 \pm 1.60	15.4 \pm 6.5	0.13 \pm 0.03
Fat	3.94 \pm 2.00	1.53 \pm 1.19	0.06 \pm 0.03	0.08 \pm 0.02
Pancreas	6.04 \pm 1.64	0.44 \pm 0.19	0.29 \pm 0.15	0.11 \pm 0.03
Muscle	5.95 \pm 1.47	0.28 \pm 0.14	0.13 \pm 0.01	0.04 \pm 0.00
Skin	18.4 \pm 6.7	0.79 \pm 0.13	0.59 \pm 0.09	0.37 \pm 0.04
Lymph Nodes	7.03 \pm 0.93	0.78 \pm 0.24	0.15 \pm 0.01	0.11 \pm 0.03
Adrenals	12.6 \pm 2.8	1.24 \pm 0.74	0.39 \pm 0.06	0.16 \pm 0.04
Brain	0.62 \pm 0.12	0.06 \pm 0.02	0.01 \pm 0.01	0
Plasma	54.1 \pm 9.8	4.06 \pm 0.20	0.57 \pm 0.05	0.16 \pm 0.01

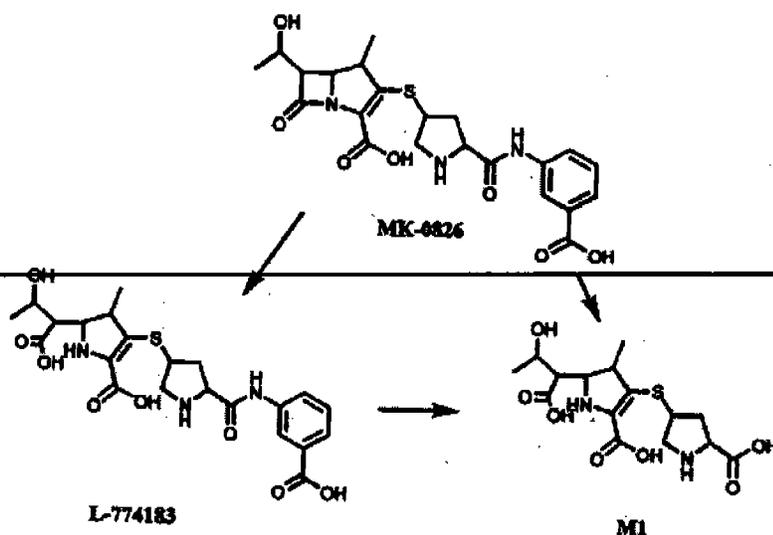
^aShown as the mean \pm SD of three animals per time point.

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Metabolism

The major metabolites of MK-0826 were an opened beta-lactam ring metabolite (L-774183) and an amide hydrolysis product (M1) as shown in the following diagram:

Proposed Metabolism Pathways of [¹⁴C]MK-0826 in Rats, Monkeys, and Humans
(Parenthetical Abbreviations Refer to Biological Matrices in Which MK-0826 and Metabolites Have Been Detected: RP = Rat Plasma, RU = Rat Urine, RB = Rat Bile, MP = Monkey Plasma, MU = Monkey Urine, HP = Human Plasma, HU = Human Urine)



(RP, RU, RB, MP, MU, HP, HU)

(RU, MU, HU)

The metabolism of MK-0826 was catalyzed by the enzyme dehydropeptidase-I as shown by the fact that L-774183 was produced in homogenates of rat kidney and lung (two tissues known to contain high levels of dehydropeptidase-I) but not in homogenates of rat liver, which does not contain appreciable quantities of dehydropeptidase-I. Also, in kidney and lung homogenates, cilastin, a specific inhibitor of dehydropeptidase-I, inhibited the formation of L-774183.

The potential for MK-0826 to influence the metabolism of other drugs was studied in a human liver microsomal preparation. The metabolism of the following drugs was measured in the presence of MK-0826 at a concentration of 240 mcg/ml (500 µM) to assess the effects on the following cytochrome enzymes:

Phenacetin	O-deethylation	1A2
Diclofenac	4'-hydroxylation	2C9
<hr/>		
<hr/>		
Testosterone	6β-hydroxylation	3A4

The microsomal metabolism of these substrates was not inhibited in the presence of MK-0826, suggesting that MK-0826 is not likely to interfere with the metabolism of drugs that are metabolized by the cytochrome P450 system.

Excretion

Urinary and fecal excretion were the major routes of elimination, as shown in the following table:

Cumulative Excretion of Radioactivity in Rats, Monkeys, and Humans After Single IV Administration of [¹⁴C]MK-0826

Species	Dose	Collection Interval (days)	Recovery of Radioactivity (% of Dose)				Feces
			Urine			Total ^a	
Rat	60 mg/kg	2	MK-0826	L-774183	M1	71.3	22.5
Monkey	30 mg/kg	3	17.4	31.9	20.0	90.7	9.3
Human	1 g	7	5.2	74.8	7.6	78.2	9.2

^aShown as sum of total radioactivity recovered in urine, including MK-0826, L-774183, M1, and several minor unknown radioactive components.

In an experiment in bile duct-cannulated rats, it was shown that about 13% of an intravenous dose was excreted into the bile within 24 hours.

Protein Binding

MK-0826 is highly bound to plasma proteins. The extent of protein binding was concentration-dependent. The compound exhibits nonlinear pharmacokinetic behavior because of saturable plasma protein binding.

The sponsor reported the unbound fractions in rat, monkey, and human plasma as shown in the following table:

In Vitro Plasma Protein Binding of MK-0826 in Rats, Monkeys, and Humans (37°C)

MK-0826 Concentration ($\mu\text{g/mL}$)	(μM)	Unbound Fraction in Plasma (%) (Mean \pm SD, n=3)		
		Rat	Monkey	Human
10	21	3.87 \pm 0.36	1.18 \pm 0.26	8.16 \pm 0.90
75	158	5.71 \pm 0.10	1.72 \pm 0.11	5.72 \pm 0.12
150	316	12.3 \pm 0.2	2.36 \pm 0.06	6.62 \pm 0.20
300	632	15.8 \pm 0.2	5.49 \pm 0.21	10.1 \pm 0
2000	4215	39.6 \pm 0.4	31.5 \pm 0.4	35.9 \pm 0.5

Maternal/Fetal Levels

In a study that was designed to investigate transfer of the compound into the fetal circulation, and into breast milk, ertapenem (700 mg/kg/day) was administered intravenously to pregnant rats in a volume of 10 ml/kg. In one group of animals, dosing occurred during gestation days 6-20. In another group, the dosing was from gestation day 6 until lactation day 14. As shown in the following two tables, it was determined that the drug entered both the fetus and the milk. There were four animals per timepoint. (997080)

Sample	Time (Min)				
	5	30	90	180	240
Maternal	1468 \pm 225	132 \pm 42	17.7 \pm 4.6	3.52 \pm 1.75	2.85 \pm 2.14
Fetal	39.5 \pm 8.4	ND	ND	ND	4.97 \pm 0.56

ND = Not determined.

Values are the Means \pm Standard Deviation

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Concentrations of MK-0826 in Rat Plasma and Milk
 Thirty minutes after dosing.
 Mean MK-0826 Concentration
 (µg/mL)

Plasma	138 ± 24
Milk	9.69 ± 1.54

Values are the Means ± Standard Deviations.

After dosing on gestation day 11, two animals exhibited seizures that lasted for approximately 10 minutes. On lactation day 8, a third animal went into seizures and subsequently died. The cause of the seizures was not determined.

Note: In the summary of toxicokinetic findings on page 6 of the sponsor's study report for this study, the dosing period was erroneously described as being from gestation day 6 through lactation day 20. It should read "from gestation day 6 through lactation day 14".

TOXICOLOGY

Unless noted otherwise, the toxicology studies described below were conducted at the sponsor's facility in Pennsylvania, and were conducted in compliance with the Good Laboratory Practice guidelines of the FDA. In most of the toxicology studies, MK-0826 was dissolved in a dilute solution of sodium bicarbonate in sterile saline, but in some of the later studies, the clinical formulation was used.

No toxicology studies in dogs were reported in this NDA. The species and strains of animals used in these studies were as follows:

Mice: CD-1

Rats: Sprague-Dawley

Rabbits: New Zealand

Monkeys: Rhesus

Intravenous injections in mice and rats were made into the tail vein, in rabbits into the ear vein, and in monkeys into the leg vein. The oral route of administration was not used in any of the toxicology studies (with the exception of the three mice dosed orally in the acute mouse study).

In many of the studies described in this section, the sponsor did not provide statistical analysis of the raw data. In some cases, means and standard deviations were also lacking.

Single-dose Toxicology Studies

Acute (single-dose) studies were conducted in mice, rats, and rabbits. No mortalities occurred in these acute studies, although one mouse, one rat, and two rabbits died in later genotoxicity, reproductive toxicity, and ocular toxicity studies.

Acute Toxicity Studies in Mice (952730) (942876) (982720)

A single oral dose of MK-0826 (500 mg/kg) was given to three female mice, and the animals were observed for seven days. There was no control group. The treatment produced no effect.

In another study, a single intravenous dose of MK-0826 (700 mg/kg) was given to three female mice, and the animals were observed for 14 days. No treatment-related effects occurred.

In a third study, a single intravenous dose of MK-0826 (2000 mg/kg) was given to six mice (three male, three female) and the animals were observed for three days. Decreased activity was observed, and lasted for about 10 minutes after dosing. There were no other effects.

Note: Although there were no deaths in this study, one mouse in a later micronucleus study, died within 48 hours of a single intravenous dose of 1000 mg/kg.

Acute Intravenous Toxicity Studies in Rats (952621) (942877)

MK-0826 (or vehicle) was administered intravenously to two groups of Sprague-Dawley rats (30 females/group) in a single dose of either 0 or 60 mg/kg. Blood was sampled for hematology measurements, prior to the dose, and at 0.5, 2, 24, and 48 hours after the dose (half of the animals at each time-point). The animals were sacrificed after 48 hours of observation. No treatment-related effects occurred (including hematology parameters).

In another study, a single intravenous dose of MK-0826 (700 mg/kg) was given to three female rats, and the animals were observed for 14 days. No treatment-related effects occurred.

Note: Although no effects were seen in this study, there were two later (reproductive and toxicokinetic) studies in which seizures/convulsions occurred in three rats in each study, following repeat doses of 700 mg/kg/day. One of these six rats died.

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Acute Intravenous Toxicity Studies in Rabbits (932785) (925012)

L-749345 was administered intravenously (ear vein) in a single dose of 225 mg/kg to three New Zealand albino rabbits (two males, one female). Two other rabbits (one male, one female) received the vehicle (_____). Blood was drawn predose, and at 48 hours post-dose for serum creatinine and urea nitrogen measurements, and the animals were then sacrificed, and the kidneys were removed.

Urea nitrogen was slightly elevated in the treated animals (as compared to controls and as compared to predose). Creatinine was slightly elevated in one treated animal. There were no other effects or microscopic changes in the kidney.

In a study conducted at _____ a single intravenous dose of MK-0826 was given to Dutch belted rabbits (two males per group) at dose levels of either 0 (water control), 20, 125, or 500 mg/kg. The animals were observed for four days, blood was collected, and the animals were then sacrificed, and the kidneys were removed.

No treatment-related changes occurred, including serum creatinine and urea nitrogen, and there were no microscopic changes in the kidney.

Note: Although there were no deaths in these studies, two rabbits died in a later ocular study, following instillation of 100 mg of L-749345 into the eye.

Repeat-dose Toxicology Studies

Multiple-dose studies were conducted in rats, rabbits (two-week duration only) and monkeys. No explanation was given as to why dogs were not studied.

Two-Week Intravenous Bone Marrow Toxicity Study in Rats (950350)

MK-0826 (or vehicle) was administered intravenously once daily to two groups of Sprague-Dawley rats (15 females/group) at doses of either 0 or 60 mg/kg/day for two weeks. The rats were six weeks old at the start of the study. The injections were made into the tail vein, after passing through a 0.22 micrometer sterilizing filter. The vehicle was an aqueous solution of neutral pH, containing sodium chloride (4 mg/ml) and sodium bicarbonate (8 mg/ml). The animals were evaluated based on observations, body weights, hematology, and gross and microscopic histopathology of only bone, bone marrow, and bone marrow smears.

There were no deaths. Mean neutrophil counts decreased from 1211 cells/mm³ in the control group to 588 cells/mm³ in the treated group. Statistical analysis of the hematology data was not presented, but there did not appear to be effects on any of the other cell types.

Intravenous Bone Marrow Toxicity and Recovery Study in Rats (950100)

This study was designed to determine if the animals would recover from the drug-induced neutropenia, following cessation of treatment. Five groups of Sprague-Dawley rats (two control, three treated) received intravenous injections of vehicle (as described above) or MK-0826 at doses of 2, 10, or 60 mg/kg/day once daily for two weeks. One of the control groups consisted of 12 rats of each sex, while the other control group, and the three treated groups contained 15/sex/group. After two weeks of dosing, the animals in the low and mid dose groups were sacrificed, while the other groups were maintained on a treatment-free recovery period of eight weeks. Hematological measurements were made.

There were three deaths in this study, all of which were control animals. Neutrophil counts decreased in response to treatment with MK-0826. The decreased neutrophil counts began to recover during the recovery period, but had not returned to control levels after the eight week recovery period. Interpretation of the hematology data (which is presented in the following table) was complicated by the lack of statistical analysis, by differences in the two control groups, and by variation in each control group over time.

Males		Control	Control	2mg/kg/day	10mg/kg/day	60mg/kg/day
NEUTROPHILS,	CELLS/MM3					
WEEK	1	1420	1113	683	831	817
WEEK	2	1676	1899	843	640	589
WEEK	3	2110	1955			943
WEEK	4	1588	2348			910
WEEK	6	1994	2162			1215
WEEK	8	1688	1742			1149
WEEK	10	1471	1754			1137

Females		Control	Control	2mg/kg/day	10mg/kg/day	60mg/kg/day
NEUTROPHILS,	CELLS/MM3					
WEEK	1	863	727	601	542	390
WEEK	2	1078	1087	600	554	474
WEEK	3	1221	1082			501
WEEK	4	1308	1494			721
WEEK	6	1367	1341			707
WEEK	8	1018	896			719
WEEK	10	864	807			645

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Intravenous Bone Marrow Toxicity and Reversal Study in Rats (952659)

This study was designed to determine if the drug-induced neutropenia could be reversed by treatment with a granulocyte colony stimulating factor.

MK-0826 was dissolved in an _____ vehicle, and dosed intravenously at 60 mg/kg/day. Commercial _____ was diluted with 5% dextrose, and administered subcutaneously at 25 mcg/kg/day. Forty Sprague-Dawley rats were divided into three groups, and treated according to the following regimen:

Group	Week 1	Week 2	Week 3
1 Control (n=10)	Vehicle	Vehicle	-

Blood was drawn for hematology on days -1 (pretest), 8, 14, and 21. The results (presented below) show that MK-0826-induced neutropenia was reversed by _____ treatment. The reversal appeared to be temporary because neutrophil counts in this group were (slightly) decreased again at day 21.

NEUTROPHILS, CELLS/MM3	Control	Drug	G-CSF
PERIOD -1	2394*	1314	1285
DAY 8	1347	546	1257
DAY 14	1730	1817	2215
DAY 21	1134	836	1052

* only seven blood specimens were obtained at this time-point, and two of those had neutrophil counts >4000. The other five specimens averaged 1672.

One-Month Intravenous Toxicity Study in Rats (940630)

MK-0826 (or vehicle) was administered intravenously once daily to Sprague-Dawley rats (15/sex/group) at doses of either 0, 30, 60, or 180, mg/kg/day for one month. The rats were six weeks old at the start of the study. The injections were made into the tail vein, and the vehicle was an aqueous solution of dilute sodium bicarbonate in sterile saline. The animals were evaluated based on observations, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic histopathology.

There were four deaths in this study (1 control, 1 low-dose, 2 mid-dose) but the deaths were not related to treatment (bleeding accidents, restraint accidents). There was a slight decrease in total white blood cell counts, and substantial decreases in neutrophils, as shown in the following table. Statistical analysis of the hematology data was not presented, but the decreases in neutrophils are clearly treatment-related. There was no effect on the bone marrow and no increase in immature neutrophils.

Males

CONTROL

30 MG/KG/DAY 60 MG/KG/DAY 180 MG/KG/DAY

LEUKOCYTES, 1,000/MM3					
WEEK	2	13.11	12.00	9.87	11.13
WEEK	4	13.47	11.98	10.54	12.23
NEUTROPHILS, CELLS/MM3					
WEEK	2	1487	604	562	557
WEEK	4	2049	719	714	729

Females

		CONTROL	30 MG/KG/DAY	60 MG/KG/DAY	180 MG/KG/DAY
LEUKOCYTES, 1,000/MM3					
WEEK	2	10.69	8.39	8.78	8.50
WEEK	4	9.48	8.62	8.71	8.67
NEUTROPHILS, CELLS/MM3					
WEEK	2	1795	502	528	361
WEEK	4	1355	534	450	360

Urobilinogen levels were increased in the urine of all ten males from the high-dose group, and in three of ten high-dose females, as compared to controls.

The following pharmacokinetic parameters were reported at the end of the study.

Gender	Dose (mg/kg/day)	Pharmacokinetics		
		AUC (µg-min/ml)	CL _r (ml/min/kg)	Terminal t _{1/2} (min)
Female	30	8492	3.53	37.3
Male	30	7779	3.86	38.2
Female	60	8875	6.76	39.7
Male	60	10075	5.96	43.5
Female	180	15811	11.4	46.6
Male	180	14409	12.5	46.9

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Three-Month Intravenous Toxicity Study in Rats (950670)

MK-0826 (or vehicle) was administered intravenously once daily to Sprague-Dawley rats (15/sex/group) at doses of either 0, 75, 225, or 675, mg/kg/day for three months. The rats were five to six weeks old at the start of the study. The injections were made into the tail vein, and the vehicle was an ~~_____~~. In four of the 30 high-dose animals, the route of administration was switched to subcutaneous during the last two to four weeks of the study, because of sores on the tail that made venipuncture difficult. The animals were evaluated based on observations, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic histopathology.

There were two deaths (one control, one mid-dose) both of which were attributed to anesthesia/bleeding accidents. Signs of vascular irritation (discoloration, flaking, sores, scabs) were observed on the tails of some animals, mainly in the high-dose group. Interpretation of the hematology data was complicated by the lack of statistical analysis, but there appeared to be some very slight decreases in leukocytes and monocytes, and substantial decreases in neutrophil counts (as shown below). Urobilinogen was increased in the urine from all treated groups. There were no other treatment-related or microscopic effects.

Males		CONTROL	75 MG/KG/DAY	225 MG/KG/DAY	675 MG/KG/DAY
LEUKOCYTES, 1,000/MM3					
WEEK	4	11.29	12.25	11.93	11.21
WEEK	8	10.25	10.22	10.02	9.67
WEEK	12	10.52	10.49	10.60	9.75
NEUTROPHILS, CELLS/MM3					
WEEK	4	1263	604	511	548
WEEK	8	1451	907	729	799
WEEK	12	1342	941	815	944
MONOCYTES, CELLS/MM3					
WEEK	4	205	191	158	151
WEEK	8	242	230	163	185
WEEK	12	271	236	200	188

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Females		CONTROL	75 MG/KG/DAY	225 MG/KG/DAY	675 MG/KG/DAY
LEUKOCYTES, 1,000/MM ³					
WEEK	4	9.36	9.26	9.03	8.69
WEEK	8	9.16	9.12	8.95	8.80
WEEK	12	8.96	8.51	7.96	8.54
NEUTROPHILS, CELLS/MM ³					
WEEK	4	787	411	409	279
WEEK	8	678	542	402	414
WEEK	12	763	471	418	747
MONOCYTES, CELLS/MM ³					
WEEK	4	160	146	108	101
WEEK	8	138	145	120	136
WEEK	12	158	167	120	133

Six-Month Intravenous Toxicity Study in Rats (990250)

MK-0826 (or vehicle) was administered intravenously once daily to Sprague-Dawley rats (20/sex/group) at doses of either 0, 60, 180, or 540 mg/kg/day for six months. The rats were five weeks old at the start of the study. The injections were made into the tail vein, and the vehicle was an aqueous solution of dilute sodium bicarbonate in sterile saline. The animals were evaluated based on observations, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic histopathology.

Organ weights were obtained for the following:

adrenals	pituitary
brain	prostate
heart	spleen
ovaries	testes
kidneys	thyroid
liver	

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The organs/tissues marked with an x were examined microscopically.

Histopathology inventory

Adrenals	X
Aorta	
Bone Marrow smear	X
Bone (femur)	X
Brain	X
Cecum	
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Harderian gland	X
Heart	X
Hypophysis	
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	
Liver	X
Lungs	X
Lymph nodes, cervical	X
Lymph nodes, mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	
Ovaries	X
Pancreas	X
Parathyroid	X
Peripheral nerve	X
Pharynx	
Pituitary	X
Prostate	X
Rectum	
Salivary gland	X
Sciatic nerve	
Seminal vesicles	
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Sternum	
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	
Trachea	
Urinary bladder	X
Uterus	X
Vagina	
Zymbal gland	

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Six rats died during the course of this study (3 low-dose, 2 mid-dose, 1 high-dose). One death in the low-dose group was attributed to an anesthesia/bleeding accident, but the causes of death in the other five animals could not be determined. Neutrophil counts were consistently lower in treated groups than in control groups, although the differences may not have been statistically significant because of variability in the data. The decreases are considered to be treatment-related, but did not progress over time. Leukocyte and neutrophil counts are presented in the following table.

Males		CONTROL	60 MG/KG/DAY	180 MG/KG/DAY	540 MG/KG/DAY
LEUKOCYTES, 1,000/MM ³					
WEEK	4	11.19 ± 1.95	11.25 ± 2.79	10.97 ± 2.89	11.44 ± 3.05
WEEK	12	9.73 ± 2.20	10.02 ± 2.27	9.72 ± 2.24	9.41 ± 1.87
WEEK	25	7.36 ± 1.83	7.75 ± 1.88	7.44 ± 1.37	7.24 ± 1.38
NEUTROPHILS, CELLS/MM ³					
WEEK	4	974 ± 379	592 ± 193	562 ± 235	548 ± 332
WEEK	12	1388 ± 443	1094 ± 432	854 ± 304	840 ± 222
WEEK	25	1204 ± 406	1116 ± 414	1067 ± 454	829 ± 215

Females		CONTROL	60 MG/KG/DAY	180 MG/KG/DAY	540 MG/KG/DAY
LEUKOCYTES, 1,000/MM ³					
WEEK	4	8.41 ± 2.32	9.47 ± 1.80	9.45 ± 2.74	9.44 ± 3.07
WEEK	12	6.94 ± 1.91	7.94 ± 2.22	7.32 ± 2.32	7.35 ± 2.58
WEEK	25	5.57 ± 1.55	5.69 ± 1.17	5.32 ± 1.38	6.02 ± 1.87
NEUTROPHILS, CELLS/MM ³					
WEEK	4	660 ± 217	440 ± 164	370 ± 140	420 ± 173
WEEK	12	847 ± 308	668 ± 319	500 ± 192	470 ± 161
WEEK	25	755 ± 370	560 ± 183	455 ± 191	439 ± 116

There were very slight decreases in total protein (accompanied by increases in the albumin/globulin ratio) in all treatment groups. The differences were not statistically significant, but appeared to be treatment-related. There were also slight increases in (urinary) urobilinogen levels that may not have been statistically significant, but appeared to be treatment-related.

No other signs of toxicity were seen in this study. Total leukocyte counts were not affected. There were no treatment-related microscopic changes in any tissue, including the bone marrow.

Another two-week study was designed to serve as a range-finding study for the reproductive studies. L-749345 was administered intravenously to female rabbits (six per group) at doses of 0, 30, 150, 300, or 700 mg/kg/day for 14 days. Blood and serum were collected and analyzed.

Decreased food consumption, diarrhea, and soft feces were seen in all treated groups. There were two deaths in the 150 mg/kg/day group. The 300 and 700 mg/kg/day groups had to be eliminated from the study because of toxicity. Neutrophils did not appear to be affected. Cholesterol, triglycerides, and transaminases were increased in the 30 and 150 mg/kg/day groups (no statistics).

Two-Week Intravenous Toxicity Study in Monkeys (931430)

L-749345 was administered intravenously to four Rhesus monkeys (2 males, 2 females, no controls) at a dose of 200 mg/kg/day for 14 days. The animals were 1-2 years old. Blood was drawn predose, and on day 11 of dosing. There was no control group.

There were no deaths, but emesis and unformed stools were seen in this study. Based on a comparison of the pre- and post-dose hematology values, there appeared to be increases in lymphocytes and eosinophils, and decreases in neutrophils, but there was considerable variation, and a lack of statistical analysis.

One-Month Intravenous Toxicity Studies in Monkeys (940640) (990890)

L-749345 was administered intravenously to Rhesus monkeys (4/sex/group) at doses of 0, 30, 60, or 180 mg/kg/day, and (in a second study) at doses of 0, 500, 750, or 1250 mg/kg/day for one month. The vehicle

The animals were 1-2 years old. Evaluations for treatment-related effects were based on observations, body weights, food consumption, ophthalmic examinations, hematology, serum chemistry, urinalysis, organ weights, and gross and microscopic histopathology.

There were no deaths. Unformed stools were seen in all treated groups, along with occasional salivation and emesis. Decreased activity and crouching behavior were observed in the 750 and 1250 mg/kg/day groups. There appeared to be increases in platelets and eosinophils, and decreases in neutrophils, but there was considerable variation, and a lack of statistical analysis. There appeared to be increases in serum triglycerides, ALT, and phosphorus (no statistics).

The following mean neutrophil counts were reported (n=8, no statistics). Note that neutrophil counts decreased between predose and week 2 of dosing in all groups, including the two control groups.

Neutrophils (cells/cubic millimeter)			
Dose (mg/kg)	Predose	Week 2	Week 4
0	4708	3033	3826
30	3602	2742	2316
60	5183	2264	2287
180	4761	2046	2513
0	5182	3011	2884
500	3291	2008	2294
750	3938	2310	2387
1250	3728	1788	2235

In the 500, 750, and 1250 mg/kg/day dose groups, liver and kidney weights (absolute and relative) were increased (as compared to controls). Microscopic changes occurred in the livers and kidneys from these groups. ~~The changes were described as hepatocytic swelling and single-cell necrosis of liver cells, and as cytoplasmic rarefaction and vacuolation in renal cortical tubules.~~

Pharmacokinetic data is shown in the tables on the next three pages.

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Dose (mg/kg/day)	Gender	Animal ID.	AUC (µg·min/ml)	CL _r (ml/min/kg)	Terminal t _{1/2} (min)*
30	Male	94-0046	79220	0.379	318
		94-0052	87657	0.342	359
		Mean	83438	0.361	338
	Female	94-0051	69021	0.435	284
		94-0057	88720	0.338	333
		Mean	78870	0.386	306
60	Male	94-0042	73370	0.818	262
		94-0058	73477	0.817	274
		Mean	73423	0.818	268
	Female	94-0047	77412	0.775	273
		94-0055	75629	0.793	282
		Mean	76520	0.784	277
180	Male	94-0050	151523	1.19	347
		94-0056	121654	1.48	319
		Mean	136588	1.34	332
	Female	94-0045	127682	1.41	307
		94-0053	122226	1.47	294
		Mean	124954	1.44	300

*Shown as harmonic mean.

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Males

Dose, mg/kg/day	Animal I.D.	Day 1			Week 4/5		
		AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$	CL_p , $\text{mL}/\text{min}/\text{kg}$	Terminal $t_{1/2}$, hr	AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$	CL_p , $\text{mL}/\text{min}/\text{kg}$	Terminal $t_{1/2}$, hr
500	98-0274	240780	2.1	5.37	225480	2.2	5.73
	99-0004	209760	2.4	5.07	215940	2.3	5.72
	98-0276	238740	2.1	5.19	246960	2.0	5.67
	99-0020	255480	2.0	5.64	233280	2.1	5.53
	Mean	236190	2.2	5.32	230415	2.2	5.66
	S.D.	19133	0.2	0.25	13113	0.1	0.09
750	98-0264	236880	3.2	4.22	242280	3.1	5.27
	99-0002	261240	2.9	4.96	262920	2.9	4.93
	98-0266	227520	3.3	4.48	239820	3.1	5.14
	99-0012	261960	2.9	4.73	259560	2.9	5.32
	Mean	246900	3.1	4.85	251145	3.0	5.17
	S.D.	17401	0.2	0.77	11780	0.1	0.17
1250	98-0272	439140	2.8	5.04	362760	3.4	5.17
	99-0006	369180	3.4	4.87	342360	3.7	4.97
	98-0278	357000	3.5	5.12	346680	3.6	4.62
	99-0014	365040	3.4	4.43	398580	3.1	4.69
	Mean	382590	3.3	4.87	362595	3.5	4.86
	S.D.	38038	0.3	0.31	25545	0.2	0.25

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Females

Dose, mg/kg/day	Animal I.D.	Day 1			Week 4/5		
		AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$	CL_p , $\text{mL}/\text{min}/\text{kg}$	Terminal $t_{1/2}$, hr	AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$	CL_p , $\text{mL}/\text{min}/\text{kg}$	Terminal $t_{1/2}$, hr
500	98-0265	233520	2.1	5.26	227160	2.2	5.78
	99-0005	220800	2.3	5.24	223440	2.2	5.75
	98-0275	172980	2.9	4.07	193680	2.6	4.91
	99-0015	212700	2.4	5.18	216360	2.3	5.29
	Mean	210000	2.4	4.94	215160	2.3	5.43
	S.D.	26125	0.3	0.58	15004	0.2	0.41
750	98-0261	292020	2.6	5.34	289260	2.6	5.72
	99-0009	288960	2.6	5.59	251040	3.0	5.64
	98-0277	321240	2.3	5.04	322620	2.3	5.61
	99-0013	275280	2.7	4.99	282540	2.7	5.41
	Mean	294375	2.6	5.24	286365	2.6	5.60
	S.D.	19332	0.2	0.28	29356	0.3	0.13
1250	98-0267	361320	3.5	4.33	382680	3.3	4.96
	99-0001	348600	3.6	4.24	373860	3.3	5.00
	98-0269	406740	3.1	3.10	400020	3.1	5.69
	99-0019	397260	3.2	4.54	389100	3.2	4.80
	Mean	378480	3.4	4.55	386415	3.2	5.11
	S.D.	27920	0.2	0.39	11013	0.1	0.39

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One-Month Intravenous Toxicity Study in Juvenile Rhesus Monkeys (979001)

This study was conducted at the California Regional Primate Research Center in 1997.

L-749345 was dissolved in bicarbonate-buffered saline and administered intravenously to 56-60 day-old rhesus monkeys (5/sex/group) at dose levels of either 0, 60, or 180 mg/kg/day for 28 days. The animals weighed from 0.7-1.1 kilograms at the start of the study. Evaluations for treatment-related effects were based on observations, body weights, ophthalmic examinations, hematology, coagulation, serum chemistry, urinalysis, organ weights, gross pathology, and microscopic histopathology. Blood samples were drawn for plasma drug concentration measurements.

In this study, neutrophil counts were reported separately for band neutrophils and segmented neutrophils. There was no effect on band neutrophils. The following data was reported for total leukocytes and segmented neutrophils in both sexes combined (mean/S. D., n=10, no statistics).

	Control	60 mg/kg/day	180 mg/kg/day
Total WBC (cells/nanoliter)			
Predose	15.5/8.0	17.3/10.3	18.4/13.4
Week 3	15.1/9.9	10.6/ 2.5	11.7/ 3.3

Neutrophils (cells/microliter)			
Predose	5507/4284	6552/2066	4878/1634
Week 3	5390/2725	3260/2254	3377/2661

The following organ weights were reported for liver/gall bladder and kidneys (mean/S. D., n=10, no statistics).

	Control	60 mg/kg/day	180 mg/kg/day
Absolute weights (grams)			
Liver/gall bladder	25.029/4.922	27.602/4.372	28.157/4.472
Kidneys	5.375/0.979	5.672/1.087	6.306/0.608
Relative weights (% of body weight)			
Liver/gall bladder	2.68/0.25	2.80/0.36	2.92/0.44
Kidneys	0.58/0.05	0.57/0.05	0.65/0.05

There were no microscopic hepatic or renal changes. No other changes that could be attributed to treatment, were observed.

Pharmacokinetic data from the three-month study is shown in the following table.

Dose (mg/kg/day)	Gender	Animal I.D.	AUC _{0-∞} (pg·min/ml)	Cl _r (ml/min/kg)	Terminal t _{1/2} (min)	
40	Female	94-0327	72540	0.551	304	
		94-0335	72781	0.550	290	
		94-0349	38043	0.689	258	
		94-0333	60985	0.656	294	
		Mean	66087	0.613	285	
		S.D.	7685	0.072	20	
	Male	94-0328	76835	0.53	299	
		94-0332	61549	0.61	252	
		94-0346	60318	0.663	238	
		94-0348	67063	0.597	292	
		Mean	66446	0.608	268	
		S.D.	7534	0.065	30	
	120	Female	94-0329	96028	1.25	263
			94-0339	94210	1.27	284
94-0341			89791	1.34	275	
94-0359			87728	1.37	256	
Mean			91930	1.31	270	
S.D.			3839	0.06	13	
Male		94-0322	122958	0.976	314	
		94-0334	96380	1.25	284	
		94-0343	97259	1.23	265	
		94-0352	88090	1.36	294	
360	Female	Mean	101172	1.30	288	
		S.D.	15100	0.16	20	
		94-0321	162077	2.22	257	
		94-0337	230694	1.36	329	
		94-0347	155598	2.31	324	
		94-0357	196795	1.83	302	
	Male	Mean	184341	1.98	300	
		S.D.	34776	0.35	39	
		94-0324	194890	1.85	274	
		94-0338	197277	1.82	271	
360	Male	94-0340	186400	1.95	304	
		94-0360	195325	1.84	290	
		Mean	193478	1.86	286	
		S.D.	4824	0.05	17	

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Six-Month Intravenous Toxicity Study in Monkeys (990260)

L-749345 was administered intravenously to Rhesus monkeys (4/sex/group) at doses of 0, 40, 120, or 360 mg/kg/day for six months. The vehicle was a mixture of ~~_____~~. The animals were 2-3 years old. Evaluations for treatment-related effects were based on observations, body weights, food consumption, ophthalmic examinations, hematology, serum chemistry, urinalysis, organ weights, and gross and microscopic histopathology.

Organ weights were obtained for the following:

adrenals	pituitary
brain	prostate
heart	spleen
ovaries	testes
kidneys	thyroid
liver	

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The organs/tissues marked with an x were examined microscopically.

Histopathology inventory

Adrenals	X
Aorta	
Bone Marrow smear	X
Bone (rib)	X
Brain	X
Cecum	
Cervix	
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Heart	X
Hyphophysis	
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	
Liver	X
Lungs	X
Lymph nodes, cervical	
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X
Pancreas	X
Parathyroid	X
Peripheral nerve	X
Pharynx	
Pituitary	X
Prostate	X
Rectum	
Salivary gland	X
Sciatic nerve	
Seminal vesicles	
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Sternum	
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	
Trachea	
Urinary bladder	X
Uterus	X
Vagina	
Zymbal gland	

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There were no deaths. Loose unformed stools, and occasional salivation and emesis were seen in all treated groups. There appeared to be decreases in neutrophils, and increases in serum phosphorus, triglycerides, and ALT (no statistics). Kidney weights (absolute and relative) were increased in all treated groups. Gross and microscopic renal changes were described. Grossly, pallor of the kidneys, especially in the renal cortex, was seen in the high-dose group. Cytoplasmic rarefaction and vacuolation, and luminal eosinophilic granularity were seen microscopically, especially in the proximal convoluted tubules, in all treated groups.

The following mean neutrophil counts were reported (n=8, no statistics). Note that neutrophil counts decreased between predose and week 4 of dosing in all groups.

Dose (mg/kg)	Predose	Week 4	Week 12	Week 25
0	6568	3041	5416	3898
40	5261	2859	2331	2048
120	5468	2224	2558	1860
360	6619	1975	2856	2739

No pharmacokinetic/toxicokinetic data was included in this study report.

Reproductive Toxicology Studies

Reproductive studies were conducted in mice and rats. An attempt was also made to study reproductive effects in rabbits, but this species could not be used because of excessive maternal toxicity (gastrointestinal effects), abortions, and mortalities.

Teratogenicity Studies in Mice (957365) (967020)

In the range-finding study, MK-0826 was administered intravenously to groups of 10 pregnant CD-1 mice at doses of either 0, 75, 150, 350, or 700 mg/kg/day during days 6-15 of gestation. On day 18 of gestation, the mice were sacrificed and the uteri were removed and opened. The uteri were examined for resorptions, and live and dead fetuses. Each fetus was weighed and received a gross external examination.

There were no treatment-related effects on the number of corpora lutea, resorptions per pregnant female, fetal survival, fetal weights, or gross abnormalities.

In the definitive study, MK-0826 was administered intravenously to groups of 25 pregnant CD-1 mice at doses of either 0, 70, 350, or 700 mg/kg/day during days 6-15 of gestation. On day 18 of gestation, the mice were sacrificed and the uteri were removed and opened. The uteri were examined for resorptions, and live and dead fetuses. Each fetus was weighed and received a gross external examination. Approximately one-half of the fetuses in each litter were dissected for a visceral examination, and all fetuses were stained with alizarin red for skeletal examination.

There was no maternal toxicity in the study. There were no treatment-related effects on the number of corpora lutea, resorptions per pregnant female, fetal survival, or gross or visceral abnormalities. Average fetal weights were decreased (5-8%) in the 700 mg/kg/day group. Also, there was a decrease in the average number of ossified sacrocaudal vertebrae in the 700 mg/kg/day group, but no other skeletal malformations.

Fertility and Reproduction Studies in Rats (997190) (957310) (957175)

In a study to evaluate male fertility, male Sprague-Dawley rats (25/group) received intravenous injections of ertapenem at doses of either 0, 175, 350, or 700 mg/kg/day for a total of eight weeks. After 29 days of dosing, the treated males were mated with untreated females. Dosing of the males continued for approximately four more weeks. The males were then sacrificed, and the testes and left cauda epididymides were collected and weighed. The testes and right epididymis were prepared for microscopic examination. The vas deferens and left epididymis were processed for sperm count and motility evaluations. On days 15-17 of gestation, the females were sacrificed and examined for corpora lutea, resorptions, and live and dead fetuses.

On the ninth day of dosing, convulsions occurred immediately after dosing in three males in the 700 mg/kg/day group. The convulsions lasted for about 30 seconds. The rate of body weight gain was also decreased in this group. ~~There were no effects on sperm count or motility, and no gross or microscopic changes in the testes or epididymides.~~ There were also no effects on mating, fertility, or fetal survival.

In a study to evaluate female fertility, female Sprague-Dawley rats (24/group) received intravenous injections of L-749345 at doses of either 0, 70, 350, or 700 mg/kg/day for 14 days before mating, during cohabitation, and until day 7 of gestation. After 14 days of dosing, the treated females were mated with untreated males. On days 15-17 of gestation, the dams were sacrificed, and examined for corpora lutea, resorptions, and live and dead fetuses.

There was no maternal toxicity, and no effects on corpora lutea, mating, fertility, or fetal survival.

In another reproduction study, pregnant Sprague-Dawley rats (10/group) received intravenous injections of L-749345 at doses of either 0, 75, 150, 350, or 700 mg/kg/day from gestation day 6 until lactation day 20. On gestation day 14, the animals were bled for hematology and serum chemistry. The animals were allowed to deliver naturally, and to rear the offspring until lactation day 21, when both dams and pups were euthanized.

Maternal toxicity in the 700 mg/kg/day group consisted of soft feces, and *increased* body weights (probably due to gastrointestinal stasis). Neutrophil counts appeared to be decreased in the 350 mg/kg/day group, but not in the 700 mg/kg/day group. There were no effects on the length of gestation, or the average number of pups per dam. There were no gross malformations in the pups.

Teratogenicity Study in Rats (957282)

MK-0826 was administered intravenously to groups of 22 pregnant Sprague-Dawley rats at doses of either 0, 70, 350, or 700 mg/kg/day during days 6-20 of gestation. On day 21 of gestation, the rats were sacrificed and the uteri were removed and opened. The uteri were examined for resorptions, and live and dead fetuses. Each fetus was weighed and received a gross external examination. Approximately one-half of the fetuses in each litter were dissected for a visceral examination; the heads of these fetuses were fixed in Bouin's solution for coronal sectioning. All fetuses were stained with alizarin red for skeletal examination.

There were no effects on the average number of live fetuses per dam, or fetal weights. There were two fetuses in a single litter from 700 mg/kg/day group with cardiovascular alterations (ventricular septal defect, cardiomegaly, situs inversus, interrupted aortic arch, and transposition of the great vessels). In another litter from 700 mg/kg/day group, there was one fetus with displaced ears, agnathia, and skeletal abnormalities (malformed cervical and thoracic vertebrae, intercostal rib, and incomplete ossification of the sternbra). There appeared to be a slight increase in the incidence of supernumerary ribs in all treated groups, and a slight decrease in the average number of sacrocaudal vertebrae in the 350 and 700 mg/kg/day groups. There were four fetuses with cleft palate in the 70 mg/kg/day group (one of these fetuses also had anasarca) but none in the 350 or 700 mg/kg/day groups.

Developmental Study in Rats (957280)

Pregnant Sprague-Dawley rats (22/group) received intravenous injections of L-749345 at doses of either 0, 70, 350, or 700 mg/kg/day from gestation day 6 until lactation day 20. The animals were allowed to deliver naturally, and to rear the offspring (F-1 generation) until lactation day 23, at which time each litter was culled to two males and two females. The following behavioral and developmental tests were performed on the F-1 generation:

Sexual maturity – females (vaginal canalization) days 28-38

Sexual maturity – males (preputial separation) days 38-48

Passive avoidance days 35 and 42

Motor activity day 70

Auditory startle response day 63

Indirect funduscopy days 45-51

Mating ability (cohabitation of non-siblings) week 11

The pups of the F-2 generation were counted, weighed, sexed, examined for external malformations, and discarded.

The only effects in the maternal (F-0) dams were statistically significant increases in body weight during lactation that occurred in all three treated groups. In the F-1 generation, there were no effects on the onset of sexual maturity, passive avoidance, motor activity, auditory response, ophthalmic examination, or mating ability. There were no effects on the number of live F-2 pups, and no external malformations in the F-2 generation.

Genetic Toxicology Studies

Bacterial Reverse-Mutation (Ames) Assay (925013)

This study was conducted at _____ in 1992.

MK-0826 was tested for the ability to induce reverse mutations in five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100). MK-0826 was cytotoxic to all strains when tested at levels of 0.32 micrograms/plate and higher. Because of the cytotoxicity, the Ames test was repeated in strains TA98 and TA100 using a "treat and plate" methodology. Using this methodology, it was possible to test at levels of up to 5 mg per plate without excessive toxicity. The tests were conducted both with and without a metabolic activation system (S9), which was obtained from the livers of rats that had been induced with _____. Positive controls were carried through the assay, although the identities of the positive controls were not given in the report.

The numbers of revertant colonies in the MK-0826 groups, were reported to be similar to those in the solvent controls, although the actual numbers were not given in the report. The positive controls gave the expected increases in mutation frequencies. It was concluded that MK-0826 was not mutagenic in this assay.

Human Cell TK6 Mutagenicity Assay (998300) (998303)

L-749345 was dissolved in normal saline, and tested for the potential to induce mutations in a human TK6 lymphoblastoid cell line. L-749345 was tested with and without a metabolic activation system, in concentrations ranging from 2 to 10 mM in the range-finding experiment, and in concentrations ranging from 4 to 10 mM in the definitive assay. The metabolic activation system (S9) was obtained from rat livers, that had been induced with phenobarbital and beta-naphthoflavone. Relative cell survival served as the criterion for a scorable dose level. Trifluorothymidine was added to select for mutations at the thymidine kinase locus. 3-Methylcholanthrene (3MC) was used as a positive control in the presence of S9, while N-nitroso-N-ethylurea (ENU) was used as a positive control in the absence of S9.

L-749345 did not increase the formation of mutant cell colonies. Relative cell survival ranged from 51-94% and was sufficient to meet the criterion of 2.5 million cells surviving at each dose level. The validity of the assay was demonstrated by the fact that both of the positive controls gave the expected increases in mutation frequency.

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Chromosomal Aberration Assays in Chinese Hamster Ovary Cells (948678) (978657)

Two batches of L-749345 with different impurity profiles were tested in these assays. A cell line obtained from Chinese hamster ovary was cultured in growth medium. L-749,345, which had previously been shown to be non-cytotoxic, was dissolved in deionized water, and added at concentrations ranging from 0.75-10 mM, with and without a metabolic activation system. The metabolic activation system (S9) was obtained from rat livers, that had been induced with phenobarbital and beta-naphthoflavone. After culturing for either 3 or 20 hours, colchicine was added to stop cell division. The cells were then harvested by trypsinization, stained, and examined microscopically for chromosomal aberrations. Cyclophosphamide was used as a positive control in the presence of S9, while mitomycin C was used as a positive control in the absence of S9.

In both studies, cell counts in treated cultures, were at least 90% of those in the controls. L-749,345 did not increase the percentage of cells with aberrations, as compared to the negative (solvent) controls. The validity of the assays was demonstrated by the fact that both positive controls gave expected results.

Alkaline Elution Assays in Rat Hepatocytes (948247) (978475)

Two batches of L-749345 with different impurity profiles were tested in these assays. Hepatocytes were obtained from the livers of (non-induced) Sprague-Dawley rats, and cultured in growth medium. Cytotoxicity was determined by cellular ATP content and resistance to trypan blue stain. L-749,345 was added in concentrations ranging from 0.25-10 mM and incubated for 3 hours. The cultures were transferred to columns that had been fitted with polycarbonate filters with a 2 micron pore size. A buffered lysis solution containing a proteinase enzyme was added, and the cells were digested. An alkaline eluting solution was added to the column, and the amount of DNA in each fraction was determined using a fluorometric assay. The amount of DNA in each fraction, and the amount remaining on the filter was plotted *versus* time. The change in the slope of the line, as compared to the control slope served as an indication of the relative number of DNA strand breaks. DMSO was the solvent control, and aflatoxin B₁ was used as a positive control.

Relative cell survival ranged from 75-89%. L-749,345 was not associated with a change in slope in either study, indicating that L-749,345 did not induce DNA strand breaks. The positive control induced strand breaks as indicated by a change in the slope of the plot.

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Micronucleus Assay in Mouse Bone Marrow (988687) (988688)

L-749,345 was administered intravenously to CD-1 mice (5/sex/group) at doses of either 0 (saline), 500, 1000, or 2000 mg/kg. Physiological saline was the solvent control, and mitomycin C was used as a positive control. The animals were sacrificed at either 24 or 48 hours after dosing, and bone marrow was harvested from the femur. The bone marrow was placed on slides, fixed in methanol, and stained with acridine orange for micronucleus assessment. The frequencies of polychromatic erythrocytes, normochromatic erythrocytes, micronuclei were recorded.

One male in the 1000 mg/kg group died; convulsions were seen in one female in the 2000 mg/kg group. There were no increases in micronuclei frequencies in L-749,345-treated groups. The positive control increased micronuclei induction as expected.

Special Toxicology/Tolerance Studies

Hemolysis Studies (934907) (944908) (954904)

Three experiments were conducted to evaluate hemolytic potential, and the results were reported in brief memo format. It was reported that no hemolysis occurred under the following conditions:

- 1) 0.1 ml of drug solution (36 mg/ml) mixed with 0.9 ml of rat, monkey, or human whole blood
- 2) drug solution (36 mg/ml) mixed with a 3% suspension of red blood cells from rat, monkey, or human (volumes not reported)
- 3) 0.1 ml of drug solutions (40 and 72 mg/ml) mixed with 0.9 ml of monkey whole blood
- 4) drug solution (72 mg/ml) mixed with a 3% suspension of monkey red blood cells (volumes not reported)

Bovine Corneal Opacity and Permeability Assay (954276)

Four bovine corneas were obtained from a meatpacking slaughterhouse, and were mounted in a specially designed, two chamber plastic holder. The corneas were incubated in a nutrient medium, and a 20% solution of L-749345 was added to one of the chambers. After four hours, corneal opacity was estimated, based on light transmission through the cornea. The nutrient medium was then removed from one of the chambers, and replaced with 1 ml of 0.5% fluorescein solution. Corneal permeability was estimated based on the amount of fluorescein detected spectrophotometrically in the opposite chamber.

According to the standard scoring system for this assay, test compounds can be classified as either mild, moderate, or severe ocular irritants. L-749345 produced only minimal effects on opacity and permeability, and was classified as mild irritant.

Ocular Irritation Study in Rabbits (954277)

L-749345 powder (100 mg) was instilled into the conjunctival sac of the left eye of three New Zealand albino rabbits (two males, one female). The eyes were not rinsed. The right eye in each animal served as an untreated control. At 0.25, 2, 24, 48, 72, and 96 hours after dosing, the cornea, iris, and conjunctiva were evaluated for opacity, redness, chemosis, and discharge according to the standard Draize scoring classification system.

Very slight conjunctival redness (lasting 24 hours) was seen in the three treated eyes. Slight discharge was observed in the three treated eyes, at the 15 minute observation only. The compound was classified as minimally irritating to the eye. Two of these three rabbits died (on days 3 and 4). The deaths were attributed to antibiotic effects on the gastrointestinal flora, that were presumed to have followed absorption of the compound through the lacrimal duct into the gastrointestinal tract.

Dermal Irritation Study in Rabbits (952729)

Hair was clipped from the backs of three New Zealand albino rabbits (one male, two females) 24 hours before drug application. L-749345 powder (500 mg) was applied to an area of ~~approximately five square centimeters on the back.~~ The site was moistened with 0.5 ml of saline, covered with gauze, and wrapped with an occlusive dressing. The animals were collared to prevent oral ingestion of the compound. After 24 hours, the dressings were removed, and the residual test substance was rinsed off with water. The sites were evaluated for edema, erythema, and eschar formation, using the standard Draize scoring classification. The animals were observed daily for one week.

There were no signs of irritation in any of the three rabbits.

Intramuscular Irritation Studies in Rabbits (962556) (972587) (972658)

Three studies were conducted in which various formulations of L-749,345 were injected into the shaved backs (sacrospinalis muscle) of New Zealand albino rabbits. A combined total of 24 rabbits (11 males, 13 females) was used in the three studies. Different areas on the back were used to compare the vehicle (1% lidocaine in normal saline), a positive control drug (cefoxitin), and the L-749,345 formulations (333 mg/ml). A single intramuscular injection (0.5 ml) was administered. Gross and microscopic observations were made at various intervals up to 30 days after dosing.

A formulation containing lyophilized L-749,345 and 1% lidocaine in normal saline, produced changes such as mild to moderate irritation, discoloration, muscle necrosis, cellular infiltration, and muscle regeneration, in most, but not all animals. The changes were reversible, and were less severe than those produced by cefoxitin.

Intravenous Irritation Study in Monkeys (960920)

A two-week intravenous toxicity study was conducted using two groups of rhesus monkeys (4/sex/group) to evaluate the potential of L-749,345 to produce venous irritation at the injection site. L-749,345 as the lyophilized sodium salt was dissolved in physiological saline (50 mg/ml), and administered via the saphenous vein at a dose of 250 mg/kg/day once daily for 14 days. The control group received injections of normal saline, according to the same treatment regimen. The animals were observed daily for two weeks, and were then sacrificed. At necropsy, only the injection sites were examined.

All of the treated animals had unformed stools, due probably to the antibiotic effect of the compound. There were no deaths, and no treatment-related changes at the injection site.

OVERALL SUMMARY AND EVALUATION

In rats, treatment-related neutropenia occurred at every dose tested (2, 10, 30, 60, 75, 180, 225, 540, and 675 mg/kg) in the multiple-dose studies. The decreases in neutrophil counts were quite substantial (being greater than 50% in some cases). There was no compensatory increase in immature neutrophils, and no effect on the bone marrow. The neutrophil decreases were not dose-related, and did not progress over time. It was shown that, upon discontinuation of dosing, neutrophil counts began to recover. It was also shown that the neutropenia could be reversed with a granulocyte-colony stimulating factor.

In monkeys treated with ertapenem (including juvenile animals), there appeared to be slight decreases in neutrophil counts, although not as large as those that had occurred in rats. However, interpretation of the primate hematology data was complicated by a lack of statistical analysis. There was a monkey study (the three-month study) where neutrophil counts were not decreased. In rabbits, the hematology data was insufficient to allow for a definitive conclusion with regard to effects on neutrophils. Other blood cell types (erythrocytes, platelets, monocytes, total leukocytes) were slightly decreased in some studies, but the incidences of these decreases were sporadic. Another marketed product (meropenem) has also been shown to cause neutropenia.

Increases in urinary urobilinogen levels occurred in rats. In rabbits, there were some slight increases in serum ALT, AST, cholesterol, triglycerides, and urea nitrogen; also some rabbits had red-colored urine that tested positive for occult blood. In monkeys, serum ALT and triglycerides were elevated, liver and kidney weights were increased, and some microscopic changes were seen in the renal proximal tubules (as described in this review). Liver and kidney weights were also increased in juvenile monkeys.

Signs typical of antibiotic-induced gastrointestinal effects in rats (e.g. cecal enlargement, diarrhea) were not described in the rat studies reported in this NDA. Gastrointestinal effects, such as diarrhea and unformed stools, were described in the rabbit and monkey studies, and these effects are thought to be due antibiotic-induced alteration of the gastrointestinal flora.

A few episodes of seizures or convulsions were reported in this NDA, although ertapenem was less potent than imipenem, in this regard. Ertapenem was not investigated for effects on respiratory rate, heart rate, or QT interval. Signs of vascular irritation at the injection sites, were observed in some animals.

In a reproductive study in mice, MK-0826 did not induce fetal abnormalities, but at the high dose (700 mg/kg/day) was associated with decreased fetal weights and decreases in the average number of ossified sacrocaudal vertebrae. In rats, there were two fetuses in one litter, with visceral (cardiovascular) abnormalities, and one fetus in another litter with skeletal abnormalities. The abnormalities occurred in the high-dose group (700 mg/kg/day), but it was not known if the effects were treatment-related, because the other 20 litters were not affected. MK-0826 did not affect fertility or development in rats.

Ertapenem was non-mutagenic in bacteria (*Salmonella*), and in a human cell line (TK6 lymphoblastoid cells). ~~Ertapenem was non-clastogenic in the Chinese hamster ovary chromosomal aberration assay, and in the mouse micronucleus test. It did not induce DNA strand breaks in the rat hepatocyte alkaline elution assay.~~

Ertapenem has not been tested in any phototoxicity, photocarcinogenicity, or carcinogenicity studies.

RECOMMENDATIONS

In the rat repeat-dose studies, neutropenia occurred at the lowest dose tested (2 mg/kg/day). This corresponds to a dose of approximately 12 milligrams per square meter of body surface area. For humans, the proposed therapeutic dose is one gram daily, or about 15-20 mg/kg/day. This corresponds to a dose of approximately 600 milligrams per square meter of body surface area. Thus, the dose proposed for humans is 10 to 50 times greater than the dose that was toxic to rats.

The effects of ertapenem in dogs (especially with regard to neutrophil counts) are unknown. It was requested that a toxicology study be conducted to investigate the effect of the compound on neutrophils in beagle dogs, but the sponsor was reluctant to conduct the requested study.

The effects of the compound on heart rates and Q-T intervals have not been reported. The intravenous administration of a new molecular entity, which has an ultraviolet absorbance maximum at 294 nanometers, may be associated with a risk of phototoxic effects. It is recommended therefore, that patients receiving this drug, be monitored for changes in the electrocardiogram, and for signs of sensitivity to sunlight.

It is also recommended that a warning be added to the label, concerning the risk of ertapenem-induced neutropenia. Other than the neutropenia warning, there is no need to change the labeling proposed by the sponsor.

Kenneth Seethaler, R.Ph., Ph.D., D.A.B.T.
Pharmacologist/Toxicologist HFD-520

cc: Original NDA 21-337
HFD-104
HFD-340

Concurrence only:

HFD-520
HFD-520/Pharm/K. Seethaler
HFD-520/MO/J. Mulinde
HFD-520/MO/J. Pohlman
HFD-520/MO/T. Smith
HFD-520/Micro/S. Altaie
HFD-520/Chem/B. Shetty
HFD-520/CSO/M. Dillon-Parker

HFD-520/R. Osterberg

HFD-520/L. Gavrilovich

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Dose, mg/kg/day	Animal I.D.	Day 1			Week 4/5		
		AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$	CL_p , $\text{mL}/\text{min}/\text{kg}$	Terminal $t_{1/2}$, hr	AUC, $\mu\text{g}\cdot\text{min}/\text{mL}$	CL_p , $\text{mL}/\text{min}/\text{kg}$	Terminal $t_{1/2}$, hr
500	98-0274	240780	2.1	5.37	225480	2.2	5.73
	99-0004	209760	2.4	5.07	215940	2.3	5.72
	98-0276	238740	2.1	5.19	246960	2.0	5.67
	99-0020	255480	2.0	5.64	233280	2.1	5.53
	Mean	236190	2.2	5.32	230415	2.2	5.66
	S.D.	19133	0.2	0.25	13113	0.1	0.09
750	98-0264	236880	3.2	4.22	242280	3.1	5.27
	99-0002	261240	2.9	4.96	262920	2.9	4.93
	98-0266	227520	3.3	4.48	239820	3.1	5.14
	99-0012	261960	2.9	4.73	259560	2.9	5.32
	Mean	246900	3.1	4.85	251145	3.0	5.17
	S.D.	17401	0.2	0.77	11780	0.1	0.17
1250	98-0272	439140	2.8	5.04	362760	3.4	5.17
	99-0006	369180	3.4	4.87	342360	3.7	4.97
	98-0278	357000	3.5	5.12	346680	3.6	4.62
	99-0014	365040	3.4	4.43	398580	3.1	4.69
	Mean	382590	3.3	4.87	362595	3.5	4.86
	S.D.	38038	0.3	0.31	25545	0.2	0.25

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/s/

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