

PHARM

REVIEW

NDA 18-998: Review and Evaluation of Pharmacology and Toxicology Data
(Original Summary; Corresp. Date 9/15/83)

Sponsor: Merck Sharp & Dohme Research Laboratories
West Point, PA 19486

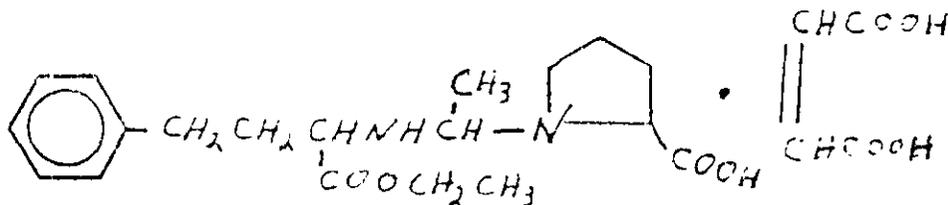
Drug:

Generic Name: Enalapril maleate

Trade Name: Vasoril

Chemical Name: (S)-1-[N-[1-(ethoxycarbonyl)-3-phenylpropyl]-L-alanyl]-L-proline, (2)-2-butene-dioate (1:1)

Chemical structure:

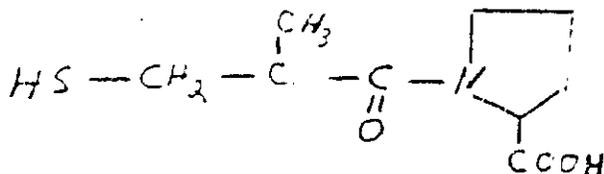


Dosage Form: Tablets, 5, 10, 20 and 40 mg

Category: Angiotensin-Converting Enzyme inhibitor

Indications: Hypertension; Congestive Heart Failure

Related Drugs:



Captopril (CapotenTM, LopirinTM)

Referenced IND: ~~XXXXXXXXXX~~ (MK-421; enalapril maleate)

Dosage and Administration:

Recommended initial dose: 10 mg once daily

Usual Dose Range: 10 to 40 mg daily in single or two divided doses.

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I. Pharmacology (Taken from sponsor's Optional Expanded Summary)

1.1. Inhibition of Angiotensin Converting Enzyme (ACE)

1.1.1. In-vitro studies

MK-422, the compound formed in vivo from the hydrolysis of enalapril (see section 2. ADME), inhibited hog plasma ACE with an IC_{50} (concentration required for 50% inhibition) of 1.25 to $1.5 \times 10^{-9}M$. MK-422 was almost 15 times more potent than captopril ($IC_{50}=2 \times 10^{-8}M$). MK-422 inhibited the ACE activity of lung, aorta, kidney and plasma of rats ($IC_{50} = 1.4-1.8$ nM). In contrast, enalapril had an IC_{50} of 1.2×10^{-6} to $5.6 \times 10^{-7}M$, because the requisite esterases which bioactivate enalapril are not present in vitro.

MK-422 and enalapril at $3.5 \times 10^{-4}M$ did not inhibit the in vitro activity of various proteolytic enzymes such as trypsin, carboxypeptidase A and B, plasmin, chymotrypsin and renin.

ACE hydrolyses angiotensin I to angiotensin II, as well as inactivating bradykinin by clearing the C-terminal dipeptides. The ability of enalapril to augment bradykinin was measured. Enalapril enhanced the contractile response to bradykinin in the guinea pig ileum by 50% at $1.8 \times 10^{-8}M$ compared to $2.4 \times 10^{-9}M$ for captopril.

1.1.2. In-vivo studies

The inhibition of the acute pressor response to angiotensin I in anesthetized and unanesthetized rats and dogs was used to assess the in vivo ACE inhibitory activity of MK-422, enalapril and captopril. The doses of these compounds which inhibited angiotensin I by 50% (ID_{50}) in sodium-replete (normal) anesthetized rats were 60.5 mcg/kg i.v. for captopril, 14 mcg/kg i.v. for enalapril and 8.2 mcg/kg i.v. for MK-422.

In anesthetized rats, single doses of MK-422 (100 mcg/kg i.v.) and enalapril (60 mcg/kg i.v.) produced a prolonged inhibition of ACE (over 2 hours), whereas captopril (300 mcg/kg i.v.) retained only about 50% of its activity at 2 hours.

In anesthetized dogs, the ID_{50} values for inhibition of the angiotensin I-induced pressor response were 80 mcg/kg i.v. for captopril, 6 mcg/kg i.v. for MK-422, and 278 mcg/kg i.v. for enalapril.

MK-422 injected into the dog renal artery dose-dependently inhibited the renal vasoconstrictor response to angiotensin I with a 65% maximal inhibition produced at 250 mg/kg. In the dog hindlimb, i.a. angiotensin-induced vasoconstrictor responses were also blocked by MK-422 ($ID_{50} = 0.8$ mg/kg i.v.).

Oral ACE inhibitory activity of enalapril was demonstrated in unanesthetized rats and renal hypertensive dogs. In rats, MK-422 (3 & 10 mg/kg p.o.) inhibited ACE activity with a duration of action at least 6 hours. Oral ID_{50} values for inhibition of the angiotensin pressor response were 0.29 mg/kg for enalapril, 0.33 mg/kg for captopril, and 2.29 mg/kg for MK-422. An

analysis of the dose-response regression lines [dose vs area under the percent inhibition curve (AUC)] showed that enalapril was 8.6 times more potent than captopril, although on an ID₅₀ basis captopril and enalapril were equipotent. In spontaneously hypertensive rats, enalapril and captopril were approximately equivalent in inhibiting ACE as measured by blockade of angiotensin I pressor responses (relative potency=1.72).

In unanesthetized renal hypertensive dogs, MK-422 inhibited angiotensin I for 6 hours at oral doses of 0.1-3 mg/kg. Enalapril was active in dogs at 0.05-1 mg/kg p.o., and inhibited the pressor response to angiotensin I for at least 6 hours at all doses. An analysis of the dose-response regression lines (AUC vs doses) indicated that in dogs enalapril was approximately 2.5 times more active than captopril by the oral route and MK-422 was approximately 2.2 times more active than captopril.

The ratios of ACE inhibitory activity by P.O./I.V. routes for MK-422, enalapril and captopril in rats and dogs were:

	P.O./I.V.	AUC 100/ID ₅₀
	<u>Rat</u>	<u>Dog</u>
Captopril	3000/60.5 = 50	250/80 = 3.1
MK-422	3000/8.2 = 366	110/80 = 3.1
Enalapril	300/14.6 = 21	110/278 = 0.3

The value in the numerator of the above ratios is the dose (mcg/kg p.o.) which was found to have an area of approximately 100 cm² as determined by polar planimetry from the angiotensin inhibitor vs time curve in unanesthetized animals. The denominator is the dose (mcg/kg i.v.) which inhibited i.v. angiotensin I pressor responses in anesthetized animals.

As an approximate index of absorption, the p.o./i.v. data demonstrate that all of the inhibitors were more active in dogs, and enalapril had the best p.o./i.v. ratio.

In dogs, plasma concentrations of MK-422 following oral administration of enalapril (0.1 and 0.3 mg/kg) or MK-422 (0.3 mg/kg) were measured and showed that the correlation between inhibition of angiotensin I and plasma MK-422 concentrations was the same for both agents. This indicates that MK-422 is the active form of enalapril in vivo.

1.2. Antihypertensive Activity

In rats fed a diet low in sodium, enalapril (1-3 mg/kg p.o.) reduced blood pressure for as long as 27 hours with certain doses. Sodium loading, by adding saline to the drinking water, substantially blocked the antihypertensive response to enalapril. Captopril was 8 times less potent than enalapril.

In sodium-deficient dogs, a pronounced lowering of blood pressure (mmHg=45) was observed with enalapril (1.0 mg/kg i.v.). In general, the hypotensive response to enalapril (or MK-422) in sodium-replete (normal) dogs was on the order of 10-15 mmHg.

In two-kidney Grollman hypertensive (2-KG) rats, enalapril lowered systolic pressure within 1/2 hour after 3 and 10 mg/kg p.o. with a maximum effect evident at 4-5 hours and a duration of action of at least 7 hours at 10 mg/kg p.o. These doses only modestly lowered systolic pressure in 1-KG hypertensive rats, a model with normal PRA.

Enalapril reduced mean arterial pressure (direct recording technique) in unanesthetized spontaneously hypertensive rats at 0.3, 1, 3 and 10 mg/kg p.o., but not in a dose-related manner. The maximum fall in mean arterial pressure averaged from 11 to 19 mmHg which was usually observed one to 2 hours after treatment. Enalapril at 3.0 mg/kg twice daily for 3 days was also antihypertensive in SH rats but by only 10-15 mmHg. A substantially greater fall in mean arterial pressure averaging 20, 41 and 34 mmHg (from the pretreatment value on Day 1) was observed in a 3-day experiment when enalapril (3.0 mg/kg p.o.) was coadministered with hydrochlorothiazide (HCTZ), 50 mg/kg p.o. for 3 days, in spontaneously hypertensive rats.

A similar enhanced antihypertensive response was observed in chronic perinephritic hypertensive dogs when enalapril, 10 mg/kg p.o., plus HCTZ (15 mg/kg p.o.) were coadministered.

In other rat experiments, enalapril (1.0 mg/kg/day p.o.) was given alone or in combination with HCTZ (50 mg/kg/day p.o.) to SH rats and its normotensive counterpart, the Wistar-Kyoto rat. Both SH and WKY rats showed antihypertensive responses to enalapril over several days.

1.3. Mechanism of Antihypertensive Action

The kidney was shown to be crucial for eliciting enalapril's antihypertensive action in SH rats. Bilateral nephrectomy abolished the effects of enalapril on blood pressure, and in the presence of a continuous angiotensin II infusion, long-term enalapril administration had very little effect on MAP in rats. These findings are consistent with a mechanism of action involving a reduction in circulating angiotensin II as part of enalapril's mechanism of action. Also, in accord with these observations because of the high PRA state, are a number of studies showing that sodium depletion either from alteration in dietary intake or with hydrochlorothiazide, substantially augmented the

antihypertensive response to enalapril. Although inhibition of plasma ACE as measured by a reduction of angiotensin I pressor responses occurred with all antihypertensive doses, the absolute fall in blood pressure with enalapril or the time at which the maximum decrement in blood pressure occurred was not related to the magnitude or duration of ACE inhibition.

Enalapril and MK-422 have been given directly into the brain of SH rats by intracerebroventricular injection (25 to 175 mcg), doses which were shown to block central angiotensin I pressor and dipsogenic responses. Neither agent reduced MAP within the first 30 minutes after injection. Other SH rat studies designed to determine whether enalapril penetrated the CNS following oral administration showed that at the time of the maximum antihypertensive response, there was no inhibition of the central pressor and dipsogenic responses to angiotensin I.

Studies related to the modification of cardiac and hindpaw sympathetic neurotransmission in diuretic-treated dogs showed that enalapril or MK-422 did not block the positive chronotropic (heart) or vasoconstrictor (hindpaw) responses to postganglionic stimulation of the cardiac and lumbar sympathetic nerves, respectively.

The role of prostaglandins in mediating the antihypertensive response to enalapril in SH rats, renal hypertensive rats (2-KG) and dogs treated with hydrochlorothiazide was investigated. Indomethacin given to rats at 1.0-1.5 mg/kg p.o. and to dogs at 5.0 mg/kg i.v. did not block the hypotensive response to enalapril in 2-KG rats and hydrochlorothiazide-treated dogs. The interaction of indomethacin by itself lowered MAP in this model. In sodium-replete dogs pretreated with indomethacin at 2.0 mg/kg p.o. or 5 mg/kg i.v., enalapril increased Na and Cl excretion, GFR, EPRF and PRA to about the same degree as was observed previously with indomethacin pretreatment.

1.4. Effects on Renal Function, Electrolyte Excretion and Plasma Renin Activity

At 1.0 mg/kg i.v., enalapril produced significant increases in sodium chloride excretion with no effect on potassium excretion in conscious sodium-replete dogs. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF) and plasma renin activity (PRA) were increased by enalapril. In adrenalectomized dogs, the increment in sodium excretion following enalapril (1.0 mg/kg i.v.) was similar to that found in sodium-replete dogs.

A significant increase in absolute renal blood flow was observed after intrarenal artery administration of MK-422 (31-125 mg/kg) in dogs on a normal sodium intake.

Enalapril had little effect on the urinary excretion of sodium or urine volume in normotensive and SH rats (0.3 to 81 mg/kg p.o.), but did have significant natriuretic activity in unanesthetized dogs at 5.0 mg/kg p.o.

Other renal studies in dogs demonstrated that the combination of enalapril, plus a subthreshold dose of HCTZ given over 3 days slightly increased urinary sodium excretion which was dose-related to the amount of HCTZ administered. A synergistic effect on plasma renin activity was also indicated.

When threshold natriuretic doses of HCTZ were given in combination with enalapril, only the combination of 10 mg/kg HCTZ plus 10 or 30 mg/kg p.o. of enalapril produced levels of sodium excretion which were greater than the sum of the effects of HCTZ plus enalapril. In this study the fall in plasma potassium which accompanied HCTZ was prevented by enalapril, 30 mg/kg. PRA was markedly increased by the combination of HCTZ plus enalapril.

1.5. Hemodynamic and Autonomic Effects

In conscious and anesthetized dogs, enalapril (1 mg/kg, p.o. or 0.1-1 mg/kg i.v.) caused a slight increase in cardiac output and a decrease in peripheral resistance. Regional blood flows were not markedly changed in conscious dogs following acute administration of enalapril.

Intravenous administration of enalapril (3 mg/kg i.v.) to anesthetized dogs, did not appreciably change the blood pressure or heart rate responses to various agents or procedures which interact with the autonomic nervous system.

1.6. Ancillary Pharmacology

Intragastric administration of enalapril or MK-422 (each 10 mg/kg) to dogs had no effect on gastrin tetrapeptide-induced gastric secretion or basal gastric secretion.

Enalapril (1-10 mg/kg p.o.) was devoid of antiinflammatory and analgesic activity in rats.

Enalapril and MK-422 (each 6-150 mg/kg i.p.) showed no activity in a battery of test procedures in mice designed to identify potential central nervous system stimulants or depressants. Similarly, enalapril (1-10 mg/kg p.o.) displayed no overt signs of central nervous system activity in squirrel monkeys.

Enalapril (1.25-10 mg/kg s.c.) and MK 422 (6.25-25 mg/kg s.c.) had no significant effect on gastrointestinal transit time of a charcoal test meal in mice.

Enalapril (2.5 and 10 mg/kg i.v.) had no significant effect on various parameters of the respiratory system (total lung resistance, dynamic compliance, tidal volume) in anesthetized dogs.

Enalapril and MK-422 (each 10 mcM) were without significant activity in radiobinding assays in rat brain for dopamine, neuroleptic, serotonin-1 and-2, alpha-2-adrenergic, beta-adrenergic, muscarinic cholinergic, GABA and benzodiazepine receptors.

2. Absorption, Distribution, Metabolism, Excretion (Taken from sponsor's Optional Expanded Summary)

2.1. Rat

In rats, peak levels of ^{14}C -enalapril radioactivity occurred in about 30-60 minutes. From plasma levels, it was estimated that about 44% of the 1 mg/kg p.o. dose was absorbed. An average of 26% of the oral dose was excreted in the urine and 72% in the feces in 72 hours. After 1 mg/kg i.v. 78% was in the urine and 19% in the feces. Thin layer chromatography studies suggest that a single radioactive component was present in the urine with an R_f similar to MK 422. Radioactivity was widely distributed in tissues at one hour and barely detectable in most tissues at 48 hours after 1 mg/kg i.v. Lung tissue had the highest levels at 24 and 48 hours. Tissue levels of radioactivity one hour after a single or four daily doses of ^{14}C -enalapril (1 mg/kg i.v.) were not significantly different, indicating that tissue accumulation of drug and/or metabolites did not occur.

In lactating rats given 10 mg/kg p.o. ^{14}C -enalapril, the drug concentration in milk averaged 0.09 and 0.36 mcg equivalents/ml at one and 4 hours after dosing, respectively. The respective average plasma levels of drug at these times were 3.64 and 0.59nmcg equivalents/ml.

2.2 Hamster

A whole body autoradiographic study of the distribution of oral ^{14}C -enalapril (5 mg/kg) was carried out in male and pregnant female golden hamsters. No radioactivity was seen in spinal cord or brain at any time after the dose. Highest levels of radioactivity were seen in decreasing order in urinary bladder, gastric contents, kidney, liver and lung at one to four hours. From 8 to 24 hours high levels were apparent in the intestinal contents, urinary bladder, kidney, liver and lung. Radioactivity was seen to decline in kidney out to 72 hours, at which time it was the only organ in which radioactivity was detectable. Although radioactivity was found transferred via the placenta to the fetus, there was no tendency for accumulation in the fetus.

2.3 Dog

In dogs, peak plasma levels of radioactivity occurred at about two hours after a 1 mg/kg oral dose. At 24 hours, levels were low but significantly higher than the I_{50} values obtained in vitro. Absorption was estimated from plasma levels to be about 64%. An average of 40% of the oral dose radioactivity was excreted in the urine and 36% in the feces in 72 hours. Following 1 mg/kg i.v., 69% was in the urine and 14% in the feces. These data suggest that biliary excretion of the prodrug or MK-422 occurred. In urine, essentially all of the radioactivity was assayed as free inhibitor. Isolation and gas chromatographic mass spectrophotometric analysis identified the active component as MK-422.

In an experiment in which bile was collected for four hours, about 22% of a 1 mg/kg i.v. dose of ^{14}C -enalapril was excreted in bile. A fraction of the radiolabeled material was intact enalapril during the first 2.5 hours, suggesting the enterohepatic recirculation of enalapril is possible.

In dogs, plasma levels and urinary excretion of radioactivity were proportional to dose over the range of 0.1 to 3.0 mg/kg p.o. Food consumed after dosing significantly delayed and diminished drug absorption.

Dog tissues obtained at five minutes after a 1 mg/kg i.v. dose of either ^{14}C -enalapril or ^{14}C -MK-422 showed that the liver contained the highest levels of radioactivity when ^{14}C -enalapril was given, and the kidney contained the highest levels when ^{14}C -MK-422 was given. Following ^{14}C -enalapril levels of radioactivity in other tissues were kidney > plasma > lung > heart; for ^{14}C -MK-422 the order was plasma > liver > lung > heart. Neither compound effectively crossed the blood-brain barrier. The section of the brain with the highest level, cerebellum cortex, contained only 3% of the plasma level of enalapril.

The physiological disposition of MK-422 was determined in dogs using unlabeled drug and converting enzyme inhibition to quantitate inhibitor. MK-422 peaked at one to two hours after 1 mg/kg p.o. Only 12% of the dose was excreted in the urine and 84% in the feces following 1 mg/kg p.o. Following 1 mg/kg i.v., 96% was in the urine and 5% in the feces. Plasma levels and these excretion data suggest that MK-422 was poorly absorbed in dogs.

Studies on the kinetics of MK-422 plasma clearance revealed that a very long terminal phase was present, which appeared to be independent of dose and which could be eliminated by concomitant infusion of captopril. These data suggest that both captopril and enalapril may be binding to the same sites (possibly to the converting enzyme).

The shape of the plasma MK-422 concentration curve over the first two to four hours was broader when enalapril was given than when MK-422 was given intravenously to dogs. In addition, the apparent volume of distribution of enalapril (0.292 ± 0.072 l/kg) was clearly greater than that of MK-422 (0.122 ± 0.032 l/kg). This suggests that the lipophilic enalapril has access to tissue sites not accessible to MK-422.

2.4 Monkey

In monkeys, plasma levels of radioactivity from [proline (UL) ^{14}C] enalapril increased steadily to six or eight hours, and then plateaued thereafter through 72 hours. In contrast, levels of inhibitor dropped dramatically after eight hours, suggesting the presence of a metabolite with a long half-life. The terminal plasma levels following dosage with ^{14}C -proline were very similar to that observed when [proline (UL) ^{14}C] enalapril was given. These data suggest that proline was a metabolite in the monkey.

Monkeys excreted 61% of the 1 mg/kg p.o. dose of [proline (UL) ^{14}C] enalapril in the urine (25%) and feces (37%) in 72 hours. Following 1 mg/kg i.v., 85% was excreted with 71% in the urine and 14% in the feces. The data suggest that biliary excretion of the drug or metabolites had occurred.

To isolate the despropyl portion of the molecule, monkeys were dosed with enalapril labeled with ^{14}C in the 2,3 phenylpropyl portion of the molecule. The des-propyl metabolite was identified by gas chromatography-mass spectrometry. It represented approximately 16% of the urine radioactivity. This mode of metabolism was not observed in the rat or dog. Monkeys dosed with [phenylpropyl-2,3- ^{14}C] enalapril showed absorption values of 78% and similar plasma profiles to those observed in dogs and rats dosed with either [proline (UL)- ^{14}C] or [phenylpropyl-2,3- ^{14}C]-labeled enalapril.

2.5 Studies of Enalapril to MK-422 Bioactivation.

In vitro studies indicate that plasma of rat, mouse, gerbil and, to a lesser extent, guinea pig were capable of the hydrolysis of enalapril to MK-422. Plasma of man, dog, rabbit, monkey, cat and hamster did not carry out the hydrolysis. Liver homogenates from rat, dog and man were capable of the bioconversion.

Evidence was obtained in vivo that post-absorptive hydrolysis occurred in the dog and monkey.

3. Toxicology

3.1 Acute Toxicity Studies

The oral LD_{50} of enalapril was 1775 mg/kg in male mice and 1767-2165 mg/kg in female mice. In rats, the oral LD_{50} was 2232-2560 mg/kg and 1821-2188 mg/kg, in males and females, respectively. Intravenous LD_{50} values ranged from 692-782 mg/kg in female mice. Toxic signs seen by either route in both species were decreased activity, ptosis, bradypnea and loss of righting reflex. In female mice, pretreatment with oral doses of HCTZ (900 mg/kg) did not significantly affect the acute toxicity of enalapril given orally one hour later. In contrast, pretreatment with 27 mg/kg p.o. enalapril slightly increased the i.p. toxicity of HCTZ.

3.2 Subchronic Toxicity Studies

One and 3 months duration studies were done in rats, dogs and monkeys given doses up to 90 mg/kg/day enalapril. In general, the high dose of 90 mg/kg/day was toxic in rats and dogs, killing many of the animals. In dogs, dosages of 30 and 90 mg/kg/day resulted in renal functional changes, such as nephrosis, increases in serum urea nitrogen and potassium and decrease in plasma sodium. Other changes at these dose levels were erosion and ulceration of the gastrointestinal tract, hypertrophy of the parathyroid, increase of osteoclasts in the bone, increases in serum GOT, GPT and alkaline phosphatase as well as hepatocellular vacuolation. Dogs that died exhibited elevation of blood glucose. Dogs given 60 mg/kg/day of enalapril and normal saline (25 ml/kg bid via gavage) for two weeks showed a marked decrease in toxicity compared to unsupplemental dogs.

In rats, serum urea nitrogen was elevated at dose levels of 30 and 90 mg/kg/day. Slight decreases in serum sodium and increases in serum potassium occurred at doses of 10 mg/kg/day and higher. Body weight gains were decreased at 30 and 90 mg/kg/day. In addition, male rats receiving 30 and 90 mg/kg/day had increases in serum alkaline phosphatase. Rats given 90 mg/kg/day enalapril and normal saline for one month did not exhibit the decreased weight gain or elevated serum urea nitrogen that occurred in unsupplemented rats. The toxicity of enalapril (90 mg/kg/day) was markedly potentiated in rats maintained on a sodium deficient diet, as evidenced by death, muscle tremor, weight loss, marked increases in serum urea nitrogen, creatinine, and potassium, renal tubular degeneration and a slight increase in serum chloride. In contrast, rats given the same dose of enalapril and a standard diet showed only a slight decrease in weight gain. A sodium deficient diet alone produced marked decrease in weight gain, slight increase in serum urea nitrogen and potassium, slight decrease in serum sodium, marked increase in plasma renin activity, renal tubular basophilia, and adrenal changes.

Monkeys tolerated up to 30 mg/kg/day enalapril with no untoward findings.

3.3 Chronic Toxicity Studies (conducted at MSDRL, West Point, PA)
3.3.1. Rat (rept. dated 2/19/82)

Laboratories, rept. dated 2/19/82)

Charles River CD rats (15/sex/gp) were given enalapril at oral (gavage) doses of 0, 10, 30, and 90 mg/kg/day for one year with a 6-month interim sacrifice (10/sex/gp). There was a dose-related increased number of deaths (1 control, 1 low, 3 mid, 4 high) during the study, although the sponsor did not consider the death to be due to drug treatment. No drug related unusual physical symptoms were reported. Starting from week 4, body weight gain decreased in all treated groups in a dose related manner. Terminal group mean weight gains were approximately 5, 8 and 16% less than control in the 10, 30 and 90 mg/kg/day females, respectively; the corresponding changes for males were 5, 10 and 18%. Ophthalmological lesions (focal retinopathy, synechia, conjunctivitis, iritis) seen in both the control and high dose rats were considered to be infection related. No drug related hematology changes were reported. Serum urea nitrogen increased with dose in each drug treated male group and in the 90 mg/kg/day female group. Serum potassium slightly increased in 90 mg/kg/day males and serum sodium and chloride slightly decreased at all dose levels. In the terminal microscopic examination, 90 mg/kg/day rats had an increased incidence of tubular basophilia of the kidneys (15/23 vs 1/21 control). The sponsor did not consider this to be drug-related since renal tubular basophilia was commonly seen in control CRCD rats at a highly variable incidence.

3.3.2. Dog (rept. date, 2/19/82)

Beagle dogs (3/sex/gp) received enalapril orally (capsule) at doses of 0, 3, 5 and 15 mg/kg/day for one year, with a 6-month interim sacrifice (2/sex/gp). There were no mortalities or drug related clinical signs. No drug-related effects on food consumption and body weight gain were reported. No drug related ophthalmologic abnormalities were observed. Electrocardiographic tracings were reported to be normal. Hematology and serum biochemistry studies were unremarkable, except for dose-dependent slight decreases in hematocrit and RBC. No drug-related histological lesions were observed.

3.4. Carcinogenicity Studies (conducted at MSDRL, West Point, PA)3.4.1. Mouse (rept. dated, 4/11/83)

A 94-week oral (gavage) study was conducted with enalapril in male CR CD-1 mice given doses of 10, 30 and 90 mg/kg/day and female CD-1 mice given doses of 20, 60 and 180 mg (base)/kg/day. Two concurrent control groups received vehicle. Based on the drug-related increase in serum urea nitrogen observed in a companion study, the high dose levels used in this study were considered to be the maximum tolerated dose. Drug administration was discontinued for either sex of any dosage group when survival dropped to 40 percent. Therefore, dosing was terminated in high dose females during week 86; for low and mid dose females during weeks 91 and 92, respectively, and for low dose males during week 93. The other male groups were dosed until week 94. No drug-related physical signs were observed. High dose females had average body weight gains approximately 10 to 30% below control until week 86 ($p < 0.05$); recovery in body weight gain to control levels occurred upon termination of dosing. Low and mid dose females had average body weight gains approximately 5 to 10% below controls during most of the study, although body weight gains were similar to controls at termination. Mid and high dose males had average body weight gains slightly less than controls (within 10%) during the study ($p < 0.05$ in high dose group only during first year). A summary of the tumor incidence data is presented below:

	Males					Females				
	CI	CII	Low	Mid	High	CI	CII	Low	Mid	High
Dose levels, mg/kg/day	0	0	10	30	90	0	0	20	60	180
No. mice starting	50	50	50	50	50	50	50	50	50	50
No. surviving to term	27	22	19	24	21	23	19	15	18	12
Percent	54	44	38	48	42	46	38	30	36	24
No. unscheduled autopsies	23	28	31	26	29	27	31	35	32	38
Percent	46	56	62	52	58	54	62	70	64	76
Time to unscheduled autopsy (weeks)	72.3	74.7	75.1	75.4	64.6	76.2	76.7	74.4	72.8	70.6
Group survival (weeks)	83.6	82.8	82.0	84.0	76.6	84.2	84.2	80.1	80.3	76.1
Total No. tumors	37	35	31	30	19	40	46	28	23	16
Tumor bearing mice	28	27	27	22	17	28	35	22	19	14
Percent	56	54	54	44	34	56	70	44	38	28
Total no. malignant tumors	17	18	12	17	10	16	14	11	11	9
Malignant tumor bearing mice	14	15	12	13	9	15	12	11	10	8
Percent	28	30	24	26	18	30	24	22	20	16

When the data were adjusted for differences in survival there was no statistically significant increased incidence of a particular tumor type in treated males and females. With respect to non-neoplastic lesions, there was a higher incidence of renal papillary mineralizations in treated animals (low 20/100, mid 30/100, high 36/100) than in controls (C_I 15/100, C_{II} 9/100). The sponsor did not consider this finding to be of biological significance.

3.4.2. Rat (rept. dated, 4/11/83)

A 106-week oral (gavage) study was conducted with enalapril in CRCD rats given doses of 10, 30 and 90 mg/kg/day. Two concurrent control groups received vehicle. Based on the decreased weight gain (see below) and increased serum urea nitrogen seen in both males and females, the high dose was considered to be the maximum tolerated dose. There were no drug-related physical signs. Male drug treated rats had average body weight gains approximately 5 to 15% below controls throughout the first 85 weeks of the study ($p < 0.05$), although body weight gains in treated rats at termination of the study were similar to the controls. In mid and high dose females, average body weight gains were up to 20% below controls during the study ($p < 0.05$). A summary of the tumor incidence data is presented below:

	Males					Females				
	CI	CII	Low	Mid	High	CI	CII	Low	Mid	High
Dose levels, mg/kg/day	0	0	10	30	90	0	0	10	30	90
No. rats starting	49	50	50	50	50	51	50	50	50	50
No. surviving to term	19	18	26	28	27	22	17	18	16	19
Percent	39	36	52	56	54	43	34	36	32	38
No. unscheduled autopsies	30	32	24	22	23	29	33	32	34	31
Percent	61	64	48	44	46	57	66	64	68	62
Time to unscheduled autopsy (weeks)	80.5	80.4	80.0	74.6	73.7	84.7	82.2	82.8	80.4	77.7
Group survival (weeks)	89.8	89.1	92.8	91.4	90.4	93.3	89.8	90.7	88.1	87.9
Total No. tumors	63	59	73	67	49	103	106	90	92	88
Tumor bearing rats	42	36	40	41	35	50	50	48	45	46
Percent	86	72	80	82	70	98	100	96	90	92
Total no. malignant tumors	16	16	15	15	13	9	15	7	13	17
Malignant tumor bearing rats	15	14	14	15	12	8	13	7	12	15
Percent	31	28	28	30	24	16	26	14	24	30

There was no evidence of a drug-related increased tumor incidence at any particular site in either males or females.

3.5 Reproduction Studies

3.5.1. Study of Fertility and General Reproductive Performance (conducted at MSDRL, West Point, PA; rept. date, 2/5/82).

Groups of CRCD rats received enalapril at oral (gavage) doses of 0, 10, 30 and 90 mg/kg/day. The males (15/gp) were treated for 70 days before mating until termination of gestation of the bred females. The females (30/gp) were treated 75 days prior to mating and throughout mating and gestation. About

half of the mated F₀ females were sacrificed on day 20 of gestation and the remaining pregnant F₀ females were allowed to delivery and nurse their offspring. The 30 and 90 mg/kg F₀ males exhibited penile lacerations which were believed to be caused by attempts to recover urine due to an apparent increase in salt appetite. No clinical signs were noted in F₀ females. During the 14 weeks of the drug treatment period, 30 and 90 mg/kg male groups gained significantly (p < 0.05) less body weight than the control groups (-24% and -19%, respectively). In females, the 30 and 90 mg/kg groups gained less weights than the control and 10 mg/kg dose groups during the pre-mating treatment period but the difference from control was significant (P < 0.05) only in the 30 mg/kg group. During the gestation period the weight gains of the drug treated female groups decreased with dose. During the lactation period, the weight gains increased in the 30 and 90 mg/kg groups.

With respect to reproduction status, there were no significant differences between control and treated groups in time to mating, pregnancy rate, number of resorptions, length of gestation or litter size.

Among F₁ offspring, the 90 mg/kg group mean fetal weight was significantly (p < 0.05) reduced. After weaning, weight gain among F₁ males of the 30 and 90 mg/kg dose groups were slightly reduced, but the reduction was statistically significant (p < 0.05) only at the 30 mg/kg dose. There were no treatment related adverse effects on postweaning weight gains of F₁ females. During the lactation period, pup mortality of the 30 and 90 mg/kg/day groups were higher than control.

Skeletal examination of F₁ fetuses delivered by Cesarean section showed dose related increases in the number of pups with skeletal variations due mainly to incomplete ossification of sternbrae and lumbar ribs. There were no skeletal variations, however, in those F₁ pups that were born normally.

In those 10 and 90 mg/kg pups selected to be nursed (4/sex/litter), there was a delay in the appearances of surface righting, auditory startle reflexes and in vaginal opening. There were no treatment-related behavioral changes in weaned F₁ offspring.

Reproductive status of F₁ females was not adversely affected by drug treatment as evaluated by pregnancy rate, postimplantation survival rate, length of gestation, neonatal F₂ pup weights, and litter size.

There were no treatment-related external abnormalities in F₂ pups.

3.5.2. Teratological Studies

3.5.2.1. Rat (conducted at MSDRL, West Point, PA; rept date, 10/8/81)

Female CRCD rats (25/gp) received enalapril at oral (gavage) doses of 0, 10, 100, 100 (plus saline), 200 and 200 (plus saline) mg/kg/day from Days 6 through 17 of gestation. The dams were sacrificed on Day 20 of gestation and the fetuses were examined for external, internal and skeletal abnormalities. There were no maternal deaths, abortions or toxic signs related to treatment. Maternal body weight gains were significantly reduced (p < 0.05) during the drug treatment period in the 100, 200 and 200 mg/kg (plus saline) groups.

Preimplantation losses were comparable between groups. There were 2 resorptions in the control group, 10 in the 10 mg/kg group, 9 in each of the 100 mg/kg and 100 mg/kg (plus saline) groups, 14 in the 200 mg/kg group, and 15 in 200 mg/kg (plus saline) group. These resorptions were from 2 dams in the control group, 7 in the 10 mg/kg group, 6 and 7 in the 100 mg/kg and 100 mg/kg (plus saline) groups, and 7 and 8 in the 200 mg/kg and 200 mg/kg (plus saline) groups, respectively. Statistical analyses revealed significant ($p < 0.05$) increases in resorptions in the 10 mg/kg as well as the two 200 mg/kg groups. The sponsor did not consider this to be drug related because of the unusually low incidence of resorptions in the control group.

Examination of the fetuses revealed no drug related external, visceral or skeletal malformations.

3.5.2.2. Rat (conducted at Nippon Merck-Banyo Co., Japan; rept date, 10/22/82)

Female Sprague-Dawley rats (25/gp) received enalapril at oral (gavage) doses of 0, 12, 120, 1200 and 1200 (plus saline) mg/kg/day from Days 6 through 17 of gestation. The dams were sacrificed on Day 20 of gestation and the fetuses were examined for external, visceral and skeletal abnormalities. There were no deaths, abortions or physical signs related to treatment. Average maternal body weight gains in the drug treated groups that were not given saline were significantly below the control ($p < 0.01$). In the 1200 mg/kg/day (plus saline) group, average weight gain was comparable to that of control. Food consumptions were markedly reduced in the 1200 mg/kg saline unsupplemented group and slightly decreased in the other drug treated groups. Reproductive status (i.e., no. implants, no. resorptions, sex ratio) was comparable among the groups. Average fetal weight was slightly, but significantly ($p < 0.05$) decreased in the saline-unsupplemented 1200 mg/kg group (3.63 gm vs 3.76 gm control).

Examinations of the fetuses revealed no drug-related external or visceral defects. The number of fetuses with the 14th rib skeletal variation was significantly ($p < 0.05$) increased in the saline-unsupplemented 1200 mg/kg group (30.2% vs 16.9% control), although the sponsor considered the difference to be due to the unusually low incidence of this variation in the concurrent control group. The historical control incidence for this variation in rats ranges from 18.1 to 45.5%.

3.5.2.3. Rabbit (conducted at MSDRL, West Point, PA; rept. date, 10/8/81)

Female New Zealand white rabbits received enalapril at oral (gavage) doses of 0, 3, 10 and 30 mg/kg/day from Days 6 and 18 of gestation. The does were sacrificed on Day 28 of gestation and the fetuses were examined for external,

visceral and skeletal abnormalities. In the 30 mg/kg/day group, 2 females died, 2 females were sacrificed in poor conditions and 2 females aborted. There were no deaths or abortions in any of the other groups.

Maternal body weight gains of the surviving rabbits during the dosing period were significantly reduced ($p < 0.05$) in 10 mg/kg/day group as compared to controls. The 30 mg/kg/day group gained only slightly less weight during the dosing period and the difference from control was not significant ($p > 0.05$).

Examination of the reproduction parameters of the surviving animals showed dose related increases in total number of resorptions (9 control, 14 low, 17 mid, 63 high) and decreases in the number of live fetuses/litter (7.0 control, 6.5 low, 5.7 mid, 2.5 high). Four of the surviving 30 mg/kg/day females had their implantations totally resorbed.

Examination of the fetuses revealed no external, visceral, or skeletal abnormalities related to drug treatment .

In oral dose range finding studies in pregnant rabbits, there was a great individual variation of sensitivity to enalapril. Mortalities occurred at 1 to 300 mg/kg/day with no apparent dose relationship, and in one study there were no deaths at 100 mg/kg/day. Abortions were observed at doses between 10 and 200 mg/kg/day and maternal body weight gains were significantly reduced at doses of 1 to 100 mg/kg/day. Significant increases in resorptions were noted at 3 and 30 mg/kg/day and there were increased dead fetuses at 1 and 30 mg/kg/day. Saline supplementation provided protection against the above findings in groups given doses up to 10 mg/kg/day, whereas no protection was provided by saline supplementation at higher dose levels.

3.5.3. Perinatal and Postnatal Study (conducted at MSDRL, West Point, PA; rept. date, 2/5/82)

Female (Fo generation) CRCD rats (20/gp) received enalapril orally (gavage) at doses of 0, 10, 30 and 90 mg/kg/day from Day 15 of gestation to Day 20 of lactation. The Fo females were allowed to deliver and rear their pups. The reproductive performance and postnatal development of the F₁ generation were also evaluated. There were no drug related maternal deaths or signs of toxicity. Dams at all dosage levels gained less weight than control during the gestation period. There were no adverse treatment-related effects on pregnancy rate, gestation length, post-implantation loss or litter size. The average pup weight at birth in the 90 mg/kg/day group was significantly ($p < 0.05$) less than that of controls.

Body weight gains of the F₁ generation pups from the drug treated groups decreased during the lactation period in a dose dependent manner. At one week after weaning, the body weights of drug treated male and female pups remained

below that of control. Male pups from the 30 and 90 mg/kg/day groups weighed significantly ($p < 0.05$) less than controls at 14 weeks after weaning. In all pups from the drug treated groups, there were delays in development as measured by the time to appearance of the righting reflex, negative geotaxis, vaginal opening, and testis descent. The sponsor considered these developmental delays to be secondary to the reduced pup weights. Behavioral tests (open field, swimming maze) were unaffected by drug treatment.

There were no malformations of F₁ offspring related to treatment.

The reproductive status of F₁ generation as revealed by pregnancy rate, time to mating, gestation length, post-implantation loss, litter size, pup weight and numbers of live and dead pups was not adversely effected.

External examination of the F₂ pups revealed no abnormalities related to drug treatment.

3.6. Mutagenicity Studies

Results of mutagenicity studies conducted with enalapril are summarized below:

Test	Laboratory	Route	Dose	Results
Reverse mutation test in <i>S. typhimurium</i> (Ames test)	MSDRI, West Point, PA	in vitro	up to 2 mg/plate	<u>Negative</u> in tester strains TA 98, TA 100, TA 1535, TA 1537 in presence of rat liver enzyme activation.
Reverse mutation test in <i>S. typhimurium</i> (Ames test)	Nomura Res. Inst., Japan	in vitro	up to 10 mg/plate	<u>Negative</u> in tester strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 in presence or absence of rat liver enzyme activation.
Reverse mutation test in <i>E. coli</i> WP2 Hcr-	Nomura Res. Inst., Japan.	in vitro	up to 5 mg/plate	<u>Negative</u> in presence or absence of rat liver activation.
Rec- assay in <i>B. subtilis</i> H17 & M45.	Nomura Res. Inst., Japan	in vitro	up to 10 mg/plate	<u>Negative</u>
Chromosomal aberration test in Chinese hamster cells	Nomura Res. Inst., Japan	in vitro	up to 20 mg/ml	<u>Positive</u> (i.e., a clastogenic effect at ≥ 10 mg/ml).
Sister Chromatid exchange test in Chinese hamster cells	Nomura Res. Inst., Japan	in vitro	up to 20 mg/ml	<u>Negative</u>
Micronucleus test in mice	Nomura Res. Inst., Japan	oral	up to 1800 mg/kg	<u>Negative</u>
Cytogenetic test in mice	MSDRI, West Point, PA	oral	up to 1946 mg/kg (1/2 LD50)	<u>Negative</u>

3.7. Drug Interaction Studies

Acute interaction studies in female mice were done with the following compounds:

Hydrochlorothiazide	Methyldopa	Aspirin
Amiloride HCl	Propranolol HCl	Indomethacin
Triamterene	Timolol maleate	Sulindac
Spiro lactone	Digoxin	Diflunisal
Furosemide	Cyproheptadine HCl	

Pretreatment with high doses of enalapril produced slight, albeit statistically significant increases, in the acute intraperitoneal toxicity of hydrochlorothiazide given one hour later. Various ratios of the oral LD₅₀ of enalapril and amiloride produced a greater mortality than expected. However, lower doses of enalapril and amiloride did not potentiate the toxicity of relatively large doses of amiloride and enalapril, respectively.

4. Package Insert

The preclinical portions of the proposed labeling represent an adequate and accurate description of the submitted data.

5. Summary and Evaluation

5.1. Pharmacodynamics

MK-422, the compound formed in vivo from the hydrolysis of enalapril, is a potent and specific inhibitor of angiotensin converting enzyme (ACE) in vitro and in vivo. MK-422 inhibited hog plasma ACE with an IC₅₀ (concentration causing 50% inhibition) of between 1.25 to 1.5 X 10⁻⁹ M. Captopril, the reference ACE inhibitor, had an IC₅₀ of 2 X 10⁻⁸ M. Thus, MK-422 was nearly 15 times more potent than captopril. MK-422 inhibited the ACE activity of lung, aorta, kidney and plasma of rats, with an IC₅₀ of 1.4 to 1.8 nM. In contrast, enalapril had an IC₅₀ of 1.2 X 10⁻⁶ to 5.6 X 10⁻⁷ M, because the requisite esterases which bioactivate enalapril are not present in vitro.

MK-422 and enalapril, at concentrations up to 3.5 X 10⁻⁴ M, did not inhibit various proteolytic enzymes such as plasmin, trypsin, chymotrypsin, carboxypeptidase A and B, and renin.

In addition to hydrolyzing angiotensin I to angiotensin II, ACE inactivates bradykinin. In in vitro experiments, enalapril enhanced the contractile response to bradykinin in the guinea-pig ileum by 50% at a concentration of 1.8 X 10⁻⁸ M. Enalapril was approximately 8 times less potent than captopril in enhancing the contractions to bradykinin.

The inhibition of the acute pressor response to angiotensin I in anesthetized and unanesthetized rats and dogs was used to assess the in vivo ACE inhibitory activity of MK-422, enalapril and captopril. In anesthetized dogs, the i.v.

ID₅₀'s were 278 mcg/kg for enalapril, 6.4 mcg/kg for MK-422 and 80 mcg/kg for captopril whereas in anesthetized rats the i.v. ID₅₀'s were 14 mcg/kg for enalapril, 8.2 mcg/kg for MK-422 and 60 mcg/kg for captopril. The difference in i.v. potency of enalapril in rats and dogs may reflect differences in the degree and rapidity with which the two species hydrolyze enalapril to MK-422.

Oral dose studies in rats and dogs showed that enalapril was more active than MK-422 in inhibiting ACE activity as evidenced by blocking the angiotensin I pressor responses. Both compounds had a longer duration of action than captopril.

Antihypertensive studies in rats and dogs showed that enalapril lowered blood pressure most effectively in those models in which the renin-angiotensin system plays a dominant role (two-kidney renal hypertension, sodium-restriction) or in low renin models made renin-dependent by diuretics (spontaneously hypertensive rat, perinephritic dogs).

In mechanism of action studies, the kidney was shown to be important for eliciting the antihypertensive action of enalapril in SH rats since bilateral nephrectomy abolished the hypotensive effects of the drug. Prostaglandins do not appear to be involved in the antihypertensive response to enalapril since indomethacin, a prostaglandin synthetase inhibitor, did not block the acute hypotensive response to enalapril in SH rats, renal hypertensive rats and HCTZ-treated dogs. In the presence of a continuous angiotensin II infusion, enalapril had very little effect on blood pressure in rats, suggesting that there were no important nonangiotensin mechanisms involved in its action. Enalapril and MK-422 did not reduce blood pressure when given directly into the brain of SH rats by intracerebroventricular injection, indicating that central inhibition of ACE is not required for antihypertensive activity. Studies in diuretic treated dogs showed that neither enalapril nor MK-422 had an inhibitory effect on sympathetic neurotransmission.

The effects of enalapril and MK-422 on renal function and electrolyte excretion were studied. In conscious sodium-replete dogs, 1 mg/kg/ i.v. enalapril increased renal blood flow and glomerular filtration rate and promotes sodium excretion. Enalapril also increased sodium excretion in adrenalectomized dogs suggesting that the natriuretic action is due to an antagonistic effect of the drug on angiotensin II-induced renal sodium reabsorption rather than due to a reduction in aldosterone release. Studies involving intrarenal artery administration of MK-422 to sodium replete dogs showed that inhibition of renal ACE is important in the renal hemodynamic response to drug.

In conscious and anesthetized dogs, enalapril (1 mg/kg p.o.; 0.1-1 mg/kg i.v.) caused a slight increase in cardiac output and decrease in peripheral resistance while regional blood flow were little affected.

Intravenous administration of enalapril, 3 mg/kg, to anesthetized dogs did not appreciably interfere with the cardiovascular responses to various agents and procedures which affect the autonomic nervous system.

Ancillary pharmacological studies showed that enalapril had no gastric antisecretory properties in rats and dogs, was devoid of central nervous system activity in mice and monkeys, and had no appreciable effect on respiratory parameters in dogs.

5.2. Pharmacokinetics/ADME

Studies with ¹⁴C-enalapril in several species of animals revealed differences in the rate of oral absorption. The estimated absorption of orally administered drug was 44% in rats, 64% in dogs, and 78% in rhesus monkeys. Peak plasma levels of radioactivity were attained within 1 hour in rats, in 2 hours in dogs and in 7 hours in monkeys. Excretions after an i.v. dose occurred mainly via the kidney, the 72-hour urinary excretions being 69%, 78%, and 71% of the administered dose in the dogs, rats and monkeys, respectively. Fecal excretions, apparently via the bile, were between 14-19% in the three species. After oral dosing, urinary excretions were 40% of the dose in dogs, 26% of the dose in rats, and 25% of the dose in monkeys and fecal excretions were 36%, 72% and 36% of the dose in the three species, respectively. In rats, dogs and hamsters, radioactivity from ¹⁴C-enalapril was distributed primarily in the liver, kidneys and lungs; radioactivity did not accumulate in any tissue upon repeated administration to rats. Neither enalapril nor MK-422 effectively crossed the blood-brain barrier, whereas radioactivity from ¹⁴C-enalapril transferred the placenta in hamsters and was incorporated into milk in rats.

The major metabolite of enalapril in dog and monkey urine was the active diacid ACE inhibitor, MK-422. In vitro studies showed that hydrolysis of enalapril to MK-422 occurred in the plasma of rat, mouse, gerbil and, to a lesser extent, guinea-pig. The plasma of man, dog, rabbit, monkey, cat, and hamster did not hydrolyze enalapril whereas liver homogenates from rat, dog and man were capable of the bioactivation. Evidence for metabolism of enalapril in addition to bioactivation to MK-422 was found only in monkeys, where approximately 16% of the urinary radioactivity was identified as despropyl MK-422.

5.3 Toxicology

One year toxicity studies were conducted with enalapril in rats at doses up to 90 mg/kg/day and in dogs at doses up to 15 mg/kg/day. No drug-related histopathological lesions were reported in either study. With the exception of a slight reduction in body weight gain, rats tolerated a dose of 10 mg/kg/day. In addition to body weight loss, higher doses (30- and 90 mg/kg/day) caused serum biochemical changes (increased urea nitrogen, electrolyte changes) and a suspected increase in mortality. Dogs tolerated doses up to 15 mg/kg/day for one year without any important toxicity. However, dogs given doses up to 90 mg/kg/day in subchronic studies exhibited renal functional changes (nephrosis, increases in serum urea nitrogen and glucose, electrolyte changes), increases in serum SGOT, SGPT and/or alkaline phosphatase accompanied by liver lesions, and mortalities. Saline supplementation was shown to ameliorate the toxicity of 60 mg/kg/day enalapril in dogs.

There was no indication of carcinogenic potential in life-time studies in mice and rats treated with maximally tolerated doses of enalapril.

The effect of enalapril on fertility and general reproductive performance was evaluated in male and female rats at dose levels of 10, 30 and 90 mg/kg/day. Males receiving 30 and 90 mg/kg/day had a reduced body weight gain. Males in these dosage groups also had penile injuries which were believed to be caused by attempts to recover urine due to an apparent increase in salt appetite. Treatment with 30 and 90 mg/kg/day reduced body weight gain of the females during gestation, but there was no drug-related effect on reproductive status. In the F₁ generation, drug treatment caused a reduced fetal weight at birth and body weight gain during lactation at 90 mg/kg/day, and an increased pup mortality during the lactation at 90 mg/kg/day, and an increased pup mortality during the lactation period at 30 and 90 mg/kg/day. There were no teratogenic effects among F₁ offspring, although a dose-related increase in the incidence of skeletal variations was seen in fetuses delivered by Cesarean section. The reproductive status of F₁ females was not adversely affected by drug treatment.

Enalapril was not teratogenic in rats at doses up to 1200 mg/kg/day or in rabbits at doses up to 30 mg/kg/day. In rabbits, however, enalapril at doses of 1 mg/kg/day or greater was shown to be embryo- or fetotoxic as evidenced by an increased number of resorptions and/or number of dead fetuses. These doses of enalapril were also maternotoxic (i.e., abortions, body weight loss, increased serum urea nitrogen, electrolyte changes, deaths), although pregnant rabbits showed marked variations in their sensitivity to the drug. Saline supplementation effectively protected against the maternotoxic and fetotoxic effects of enalapril at doses up to 10 mg/kg/day, although no protection was provided by saline supplementation at 30 mg/kg/day. In rats, decreased fetal weights occurred at 1200 mg/kg/day, but did not occur at this dosage level in saline-supplemented animals. Maternal body weight gains were reduced at doses as low as 12 mg/kg/day, but not in saline-supplemented rats given up to 1200 mg/kg/day.

In a peri- and postnatal study in rats, maternal weight gains were reduced during gestation at doses of 10 mg/kg/day and higher, although there was no treatment-related effect in reproductive status. Pup body weight at birth was reduced in the 90 mg/kg/day group, and pup body weight gains were reduced at dose levels of 10 mg/kg/day and higher, causing a secondary delay in postnatal reflex and sexual development. The reproductive status of the F₁ generation was unaffected by drug treatment, nor were there any drug-related external abnormalities in the F₂ pups.

The genotoxic potential of enalapril was evaluated using a battery of in vitro and in vivo tests. Negative results were obtained in microbial/mammalian microsomal mutation assays, the Rec-assay, a sister chromatid exchange test, and the micronucleus test in mice. A positive test (i.e., a clastogenic effect) occurred in an in vitro chromosomal aberration test in Chinese hamster cells, but only at relatively high concentrations (≥ 10 mg/ml). In an in vivo cytogenetic assay in mice, however, negative results were obtained at single oral doses up to 1946 mg/kg (1/2 the LD₅₀).

6. Recommendation:

NDA 18-998 is approvable with respect to the preclinical pharmacology and toxicology portions.

7. Pharmacology and Toxicology Sections of Summary Basis of Approval (Prepared by Sponsor)

Pharmacology

Studies Related to the Primary Therapeutic Activity.

1. Inhibition of ACE

Enalapril is the monethyl ester of the systemically active angiotensin converting enzyme (ACE) inhibitor, enalaprilat (MK-422). Following oral administration enalapril is bioactivated by hydrolysis to enalapril which is the active ACE inhibitor. Hog plasma ACE is inhibited by enalaprilat and enalapril with IC_{50} 's of $1.25-1.5 \times 10^{-9}$ M and $1.2 \times 10^{-6}-5.6 \times 10^{-7}$ M, respectively. Enalaprilat, in addition to inhibiting plasma ACE, also inhibited tissue ACE (lung, aorta, kidneys) of rats with an IC_{50} of 1.4 to 1.8 nM. In anesthetized rats and dogs, enalaprilat was about 7 and 12 times, respectively, more potent than the reference inhibitor, captopril, in inhibiting the pressor response to exogenously administered angiotensin I. In general, the duration of action of intravenous enalaprilat was longer than that of captopril in rats and dogs. The computation of an ID_{50} value for prodrug inhibitor enalapril in rats and dogs has been estimated, but it may not be especially meaningful because the generation of the active form of the drug in plasma is likely to be different from animal to animal and the rate of hydrolysis to the active form (enalaprilat) is species-dependent.

2. Antihypertensive Activity

From oral studies in rats and especially in dogs, enalapril was more active than enalaprilat in blocking angiotensin I pressor responses. Both inhibitors had a longer duration of action than captopril.

A number of antihypertensive studies showed that enalapril lowered blood pressure most effectively in those models which are characterized by a high level of plasma renin activity (two-kidney Grollman, salt restriction) or in low renin models made renin-dependent by diuretics (SH rats).

3. Mechanism of Antihypertensive Action

A number of mechanism of action studies were performed. The role of prostaglandins in the antihypertensive response to enalapril was discounted because indomethacin, a prostaglandin synthetase inhibitor, did not attenuate the acute hemodynamic effects of the compound. In rats

which were infused continuously with angiotensin II over ten days, enalapril had very little effect on blood pressure, suggesting that there were no important non-angiotensin mechanisms involved in its action. In mature SH rats, enalapril did not acutely reduce blood pressure following an intracerebrovascular injection, indicating that central inhibition of ACE is not crucial for blood pressure lowering. Taken collectively, the weight of available data indicates that enalapril lowers blood pressure as a consequence of peripheral blockade of the formation of angiotensin II.

4. Effect on Electrolytes and Renal Function

The effects of enalapril on electrolyte excretion and renal function may be a contributing factor to its anti-hypertensive efficacy. Enalapril increases renal blood flow and glomerular filtration rates and promotes sodium excretion. The mechanism of action of the natriuresis involves a modulation of aldosterone biosynthesis via angiotensin II on the adrenals, a change in renal function and probably an antagonism of the anti-natriuretic effect on angiotensin II.

Studies Relating to Secondary Pharmacologic Action

With regard to other pharmacological properties, there is no direct evidence for any mechanism of action of enalapril other than specific inhibition of ACE, but the precise site where enalapril decreases the levels of angiotensin II (or increases the levels of bradykinin) has not been identified.

1. Cardiovascular and Autonomic Actions

Other pharmacological experiments revealed that enalapril, administered intravenously, did not antagonize or enhance several standard pressor or depressor agents which interact with the autonomic nervous system, although the inhibitor slightly reduced mean arterial pressure. A central anti-hypertensive effect was not demonstrated in anesthetized rats via an intracerebroventricular infusion.

In conscious and anesthetized dogs, enalapril 1.0 mg/kg p.o. or 0.1 to 1.0 mg/kg i.v., caused a slight increase in cardiac output and a decrease in peripheral resistance. Regional blood flows were not markedly changed in conscious dogs following acute administration of enalapril.

2. Gastrointestinal Actions

A number of ancillary pharmacological experiments have demonstrated that enalapril has no gastric anti-secretory or gastric irritant properties in dogs and rats.

3. Central Nervous System and Other Actions

Enalapril had no behavioral or central nervous system properties in mice and monkeys in a battery of test designed to uncover a variety of central nervous system properties. Enalapril had no significant activity on respiratory parameters in dogs and had no anti-inflammatory or analgesic properties in rats.

Toxicity

1. Acute Toxicity:

The oral LD₅₀ for enalapril in the mouse and rat is approximately 2 gm/kg. The intravenous LD₅₀ for enalapril in the female mouse is approximately 750 mg/kg. Regardless of the route, sex, or species, signs were generally similar consisting of ptosis, decreased activity, and bradypnea. Acute studies in fed versus fasted mice showed similar signs of drug effect, except that deaths generally occurred slightly later in the fed mice. In dogs, a single dose of 200 mg/kg caused deaths after three or four days.

Acute interaction studies in female mice with enalapril and hydrochlorothiazide, amiloride HCl, triamterene, spironolactone, furosemide, methyl dopa, propranolol HCl, timolol maleate, digoxin, cyproheptadine HCl, aspirin, indomethacin, sulindac, and diflunisal produced no interactions which would be of clinical significance. Pretreatment with enalapril produced statistically significant increases in the acute intraperitoneal toxicity of hydrochlorothiazide given one hour later; however, these changes are slight and at very high doses. Enalapril given with amiloride (a potassium sparing diuretic) at various ratios of the oral LD₅₀ of each produced a greater mortality than expected. However, at pharmacologically active doses of either compound, no potentiation of toxicity of relatively large doses of the second compound occurred, suggesting no toxicologic hazard.

2. Subacute and Chronic:

Rats were given 10, 30 or 90 mg/kg/day of enalapril orally by gavage for up to one year. Slight decreases in body weight gain occurred at all dose levels and moderately elevated serum urea nitrogen values occurred at the two highest dosage levels. Very minor serum electrolyte changes occurred which may reflect the pharmacologic suppression of aldosterone. No drug-induced histological changes were seen in the rats. Physiologic saline in place of drinking water prevented the BUN elevation changes produced by enalapril in rats given 90 mg/kg/day, while a diet deficient in sodium greatly potentiated toxicity of this dose in rats. Toxicity seen in rats given enalapril and a sodium deficient diet consisted mainly of weight loss, marked increase in BUN, creatinine, and potassium, renal tubular degeneration, and death.

Dogs given up to 15 mg/kg/day of enalapril orally for one year showed no drug-induced changes. However, when dogs were given 30 mg/kg/day or 90 mg/kg/day of enalapril for up to 3 months, toxic changes occurred consisting of increases in BUN and glucose, decreases in serum chloride, renal tubular degeneration, and death. The dogs given 90 mg/kg/day, a highly toxic dose,

also exhibited increases in SGOT, SGPT, and/or alkaline phosphatase activity accompanied by very slight to slight fatty changes in the liver and hepatocellular necrosis.

In another study of 2 weeks duration, physiologic saline given concomitantly by gavage to dogs receiving 60 mg/kg/day of enalapril, markedly decreased the toxicity compared to unsupplemented dogs.

Monkeys given up to 30 mg/kg/day of enalapril for one month showed no drug-related changes.

3. Mutagenicity and Tumorigenicity:

Enalapril was negative in reverse mutation assays with Salmonella typhimurium and Escherichia coli, Rec-Assay with Bacillus subtilis and in the sister chromatid exchange using cultured mammalian cells. Enalapril was also negative in the in vivo micronucleus test with mice and did not produce chromosomal aberrations in mouse bone marrow cells when given at the highest possible in vivo dose.

A clastogenic effect seen with enalapril in an in vitro chromosomal aberration test was considered to have no biological significance because the concentration required to produce this change (10 mg/ml) is highly unlikely to occur under in vivo conditions. This concentration is also several fold above the drug concentration obtained in using therapeutic doses in humans. No chromosomal aberrations were seen in the assay at concentrations of 5 mg/ml.

Enalapril was not carcinogenic when administered by gavage for up to 94 weeks at maximally tolerated doses of up to 90 mg/kg/day for male mice and up to 180 mg/kg/day for female mice. Dosing was terminated in the high dose female mice in the 86th week because of reduced survival. Rats given up to 90 mg/kg/day for gavage for 106 weeks also did not reveal any carcinogenic potential for enalapril.

4. Teratogenic and Reproductive Studies:

Enalapril at oral doses up to 1200 mg/kg/day did not produce embryoletality or teratogenicity in rats when given during the period of organogenesis. Decreased average fetal weight occurred at 1200 mg/kg/day, but not at the next lowest dose tested of 120 mg/kg/day. The decreased average fetal weight at 1200 mg/kg/day was prevented if the pregnant animals were supplemented with physiologic saline.

Enalapril was not teratogenic in rabbits when given orally at doses up to 30 mg/kg/day from days 6 to 18 of gestation. Maternal and fetal toxicity were, however, noted at doses as low as 1 mg/kg. Saline supplementation prevented this toxicity with doses of enalapril up to 10 mg/kg but not at the dose of 30 mg/kg.

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In reproductive studies (late gestation and lactation and/or fertility studies) oral doses of 10, 30 or 90 mg/kg/day of enalapril in rats produced slightly decreased maternal weight gain at all doses, reduced mean Day 1 postpartum pup weight at 90 mg/kg/day, decreased weight gain of F₁ pups during lactation with secondary delayed postnatal development at all doses, an increased number of deaths during lactation at 30 and 90 mg/kg/day and a reduced weight gain in the postweaning period in males given 30 and 90 mg/kg/day. There were no adverse effects on the reproductive performance of male or female rats.

Michael A. Commarato 6/13/84
Michael A. Commarato, Ph.D.

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