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Preclinical Profile of Zofenopril: An Angiotensin Converting Enzyme Inhibitor with Peculiar Cardioprotective Properties

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INTRODUCTION

The discovery of captopril, the prototype of orally active angiotensin-converting enzyme inhibitors (ACEIs), represented a major breakthrough in the treatment of cardiovascular diseases. Currently, captopril has four important indications: hypertension, congestive heart failure, acute myocardial infarction, and diabetic nephropathy. After the discovery of captopril, several new ACEIs were developed and introduced into medical practice. These new ACEIs are neither chemically nor pharmacologically identical; they differ in their chemical structure, functional groups (sulfhydryl in captopril, carboxyl in enalapril, or phosphinyl in fosinopril), active moiety (some are prodrugs), potency, ancillary pharmacology, and pharmacokinetics. These and other important characteristics differentiate ACEIs and influence their ability to inhibit the enzyme in various organs. Since ACEIs appear to work by inhibiting angiotensin-converting enzyme (ACE) in critical tissues, tissue selectivity is one of the most important properties that varies with the individual ACEIs.

An important question is whether different tissue-selectivity profiles of ACEIs in animal experiments are clinically relevant. Although the clinical relevance is not yet firmly established, the emerging evidence indicates that some differences among ACEIs are clinically significant (4,6,28,43).

The latest ACE inhibitor to reach the European market is zofenopril calcium. By February 1999, it was registered in all 15 European Community countries. The goal of this review is to summarize all available preclinical data on zofenopril with a particular emphasis on its physicochemical properties and pharmacodynamic/pharmacokinetic characteristics, including its high lipophilicity and selective cardiac ACE inhibition, as well as the free radical scavenging properties of its sulfhydryl group. These properties are re-

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sponsible for the cardioprotective activity of zofenopril and its high potential in the prevention and therapy of cardiovascular diseases. The benefits of zofenopril have been demonstrated in patients with acute myocardial infarction. In a placebo-controlled study, zofenopril, given daily for 6 weeks, reduced the risk of severe chronic heart failure and death in patients with acute myocardial infarction by 46% and 25%, respectively. These beneficial effects were maintained for at least 1 year (1).

CHEMISTRY

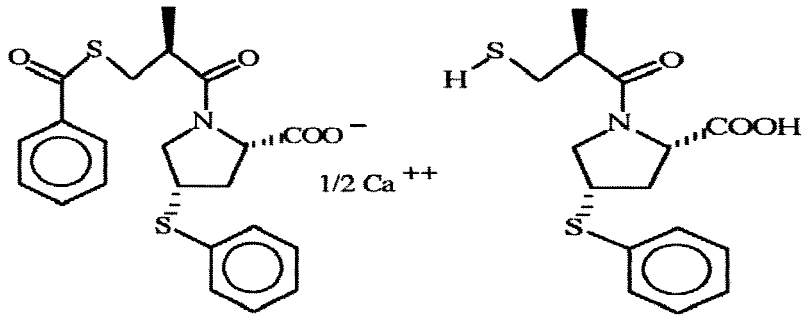
Zofenopril calcium, [1(*S*), 4(*S*)]-1(3-mercapto-2 methyl-1-oxopropyl) 4-phenyl-thio-*L*-proline-S-benzoylester (Fig. 1), formerly SQ 26,991 or MEN 8029, is a new sulfhydryl-group–containing ACE inhibitor. Tradenames of zofenopril are Zofenil® and Bifril®.

Zofenopril is a prodrug, that is deesterified to the active inhibitor, the sulfhydryl group containing compound, zofenoprilat (Fig. 1). Zofenopril calcium is a chemically stable, white crystalline powder, with a melting point higher than 250°C and a molecular weight of 448.59. The water solubility of zofenopril is 0.3 mg/mL and the pH of the saturated solution is 6.7. It is slightly soluble in dimethyl formamide and methanol and practically insoluble in isopropanol, 1-butanol, acetone, acetonitrile, and ethyl acetate. As shown in Table 1, fosinopril, zofenopril, and their respective active metabolites are highly lipophilic in comparison to other inhibitors of ACE (33).

PHARMACOLOGY

In vitro ACE inhibition

The ACE-inhibitory activity of zofenopril was first assessed in a rabbit lung extract. It was expressed as the concentration required to inhibit histidyl-leucine formation from the synthetic substrate, hippuryl-histidyl-leucine. In guinea pig ileum zofenopril inhibited the contractile response to angiotensin I and augmented the contractile response to bradykinin (35). The EC_{50} of zofenoprilat was in the nanomolar range (1–8 nM), so that zofenoprilat was 3–8 times more potent than captopril. The prodrug zofenopril was active by itself in



Zofenopril **Zofenoprilat**

FIG. 1. Structural formula of zofenopril calcium and its active metabolite zofenoprilat.

TABLE 1. *Comparison of octanol-water distribution coefficients of ACE inhibitors determined at pH 7 (modified from ref. 33)*

Compound	Distribution Coefficient
Captopril	0.004
Zofenoprilat	0.22
Zofenopril	3.5
Enalaprilat	<< 0.001
Enalapril	0.07
Ramipril	1.12
Ramiprilat	0.011
Lisinopril	< 0.001
Fosinoprilat	0.33
Fosinopril	≈500

the same *in vitro* models (EC_{50}: 6–80 nM), being 2–3 times less potent that captopril. In both assay systems, zofenopril was apparently rapidly converted to zofenoprilat.

Zofenoprilat has been also tested for its ACE inhibitory activity in homogenates of aorta, brain, heart, lung, kidney, and serum of spontaneously hypertensive rats (SHRs) (8,9). As shown in Table 2, zofenoprilat inhibited ACE in these tissues with a very similar potency (IC_{50}: 0.8–2.8 nM). It was approximately six times more potent than captopril, twice as potent as enalaprilat for fosinoprilat, but three times less potent than ramiprilat. Also, its prodrug ester zofenopril, when tested at the same experimental conditions, yielded IC_{50} values very similar to those of zofenoprilat in all tissues (but not in serum), indicating that the prodrug is completely cleaved and forms the active inhibitor in these tissues (Table 2). This property of zofenopril differentiates it from other prodrugs with ACE inhibitory properties (ramipril, fosinopril, enalapril) that are activated significantly, although incompletely, only in serum and kidney.

In vivo ACE inhibition

The ACE-inhibitory activity was assayed in conscious rats, dogs, and monkeys by evaluating the pressor response to angiotensin I following oral administration of zofenopril (11). Zofenopril (0.03–0.6 mg/kg) induced a dose-dependent inhibition of this response and, on the molar basis, was six to ten times more potent than captopril. The specificity of zofenopril and zofenoprilat actions was demonstrated in vitro and in vivo: these drugs did not antagonize the effects of angiotensin II or other spasmogens (11).

Tissue ACE inhibition

The ability of zofenopril to inhibit cardiac tissue ACE was first evaluated *in vitro* in comparison with several other inhibitors. Isolated rat hearts were perfused with Krebs-Henseleit solution containing different concentrations of the ACE inhibitors (15). As shown in Fig. 2, concentration-response curves for various "free" inhibitors differed substantially from each other; zofenoprilat and fosinoprilat were at least one order of magnitude more potent than the others. Even larger and more significant differences were observed when prodrug esters were used. Their effectiveness in inhibiting cardiac ACE depended on the uptake of a prodrug by the heart as well on its hydrolysis to the active inhibitors by esterases in the cardiac tissue.

TABLE 2. *IC_{50}s of ACE inhibitors in uncentrifuged tissue homogenates and relative rates of activation of prodrug esters (modified from ref. 8)*

		IC_{50} (nM)		
		Active Inhibitor	Prodrug Ester	
Drug	Tissue		0 min Preincubation	60 min Preincubation
Captopril	Aorta	13.0		
	Brain	8.8		
	Heart	7.7		
	Kidney	7.7		
	Lung	11.0		
	Serum	9.9		
Zofenopril	Aorta	1.6	74	1.1
	Brain	0.8	24	0.5
	Heart	2.8	59	0.9
	Kidney	1.0	22	3.1
	Lung	1.8	93	1.8
	Serum	2.3	360	71
Enalapril	Aorta	9.0	6500	290
	Brain	3.6	8400	3700
	Heart	2.6	4500	1600
	Kidney	2.8	860	22
	Lung	2.8	6800	1400
	Serum	2.4	810	22
Ramipril	Aorta	0.6	720	78
	Brain	0.6	1300	140
	Heart	0.7	810	16
	Kidney	0.9	150	1.7
	Lung	0.7	700	15
	Serum	0.5	16	0.7
Fosinopril	Aorta	2.9	990	30
	Brain	2.4	3100	130
	Heart	3.4	3400	390
	Kidney	2.2	41	8
	Lung	2.5	3700	53
	Serum	20.0	400	38

Table 3 summarizes the relative efficiencies with which the ACE inhibitors were taken up by the heart when perfused either as "free" inhibitors or as prodrug esters. The efficacy of the uptake was compared with that of fosinoprilat (its efficacy = 100%). The relative efficiency was calculated as the ratio of prodrug-/free-inhibitor uptake efficacy multiplied by 100. The relative rates of prodrug hydrolysis by the heart are ratios of the apparent IC_{50} values of the prodrug ester to that of free inhibitory form of the drug after incubation of each compound for 60 min with rat heart homogenate. As Table 3 indicates, fosinoprilat and zofenoprilat were taken up the by heart far more efficiently than lisinopril, enalaprilat, or ramiprilat. Among the prodrug esters, which depend on both uptake and ester hydrolysis to inhibit cardiac ACE, zofenopril was the most efficient, being 200 times superior to enalapril and 1500 times superior to ramipril. When the efficiency of the uptake was compared for each pair of a prodrug and a free inhibitor, the importance of the rate of prodrug hydrolysis by the cardiac tissue became obvious. Zofenopril was even more efficient at delivering the inhibitor than the free inhibitor itself, while all other prodrugs were less efficient. Zofenopril is taken up by the heart more readily than the free sulf-

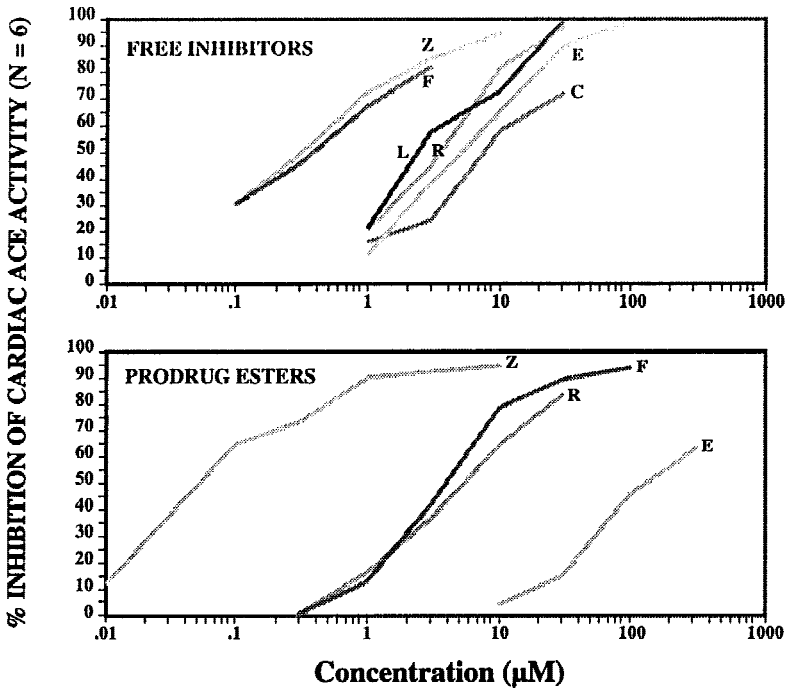


FIG. 2. Relationship between the concentration of ACE-inhibitory drugs perfused through isolated rat hearts and the inhibition of cardiac tissue ACE. Hearts were perfused with the active inhibitory forms (upper panel) or prodrug ester precursors (lower panel) of the following ACE inhibitors: captopril (C), enalapril (E), fosinopril (F), lisinopril (L), ramipril (R), and zofenopril (Z) (modified from ref. 17).

hydryl compound and is rapidly hydrolyzed to inhibit ACE. The tissue ACE-inhibitory activity of zofenopril was also evaluated ex vivo in SHRs and compared with that of other inhibitors. The selected doses were normalized for differences in the molecular weight and inherent inhibitory potency to compare tissue distribution independent of differences in potency; they produced generally similar antihypertensive effect in SHRs (i.e., 20%

TABLE 3. *Relative distribution into rat heart tissue of ACE inhibitors perfused as free inhibitors or as esterified prodrug precursors*

Drug Perfused	Efficacy of Inhibitor Uptake		Relative Efficiency: Prodrug vs. Free Drug %	Relative Rates of Prodrug Hydrolysis by Heart Tissue* % vs. zofenopril
	Free Inhibitors	Prodrugs (% vs. fosinoprilat)		
Fosinopril	100	9.2	9.2	0.9
Zofenopril	61	310	510	100
Captopril	15			
Lisinopril	6.9			
Enalapril	6.5	0.2	3.1	0.2
Ramipril	2.2	1.5	68	4.4

* Ratio of the apparent IC_{50} value of the prodrug ester to that of the free-inhibitory form of the drug after each compound was incubated for 60 min with rat heart homogenate (modified from ref. 17).

lowering in arterial pressure). Table 4 summarizes the time course of tissue ACE inhibition by various ACE inhibitors. All drugs produced a nearly complete inhibition of serum ACE at the early time points, confirming that doses used are nearly equivalent with respect to maximal inhibitory activity in the blood and that differences observed in other tissues are due to selective tissue distribution rather than to differences in inhibitory potency. In the aorta, the inhibitory activity of zofenopril as well as those of ramipril and lisinopril persists for more than 4 days, while the other compounds are definitely shorter acting. The long duration of the inhibitory action of zofenopril on vascular ACE correlates well with its long-lasting antihypertensive action. However, the largest difference was found in the cardiac ACE inhibition, where zofenopril produced a striking and long-lasting inhibition. Captopril and fosinopril were also active, but had shorter duration of action, while the other compounds had only slight and transient inhibitory effects (Table 4).

Another study (35) examined the inhibitory properties of 10 mg/kg zofenopril in rat tissues by means of quantitative in vitro autoradiography using [^{125}I]351 A, a tyrosyl

TABLE 4. *Inhibition of tissue ACE as a function of time after administration of equivalent oral doses of different ACE inhibitors to SHR (modified from ref. 8)*

Drug (dose, mg/kg) Time After Dosing	% ACE Inhibition			
	Aorta	Heart	Kidney	Serum
Captopril (30)				
1 h	86*	91*	86*	98*
8 h	54*	28*	43*	22†
1 d	31*	7	25*	–39*
2 d	8	–3	4	–41*
Zofenopril (10)				
1 h	77*	91*	87*	98*
8 h	48*	59*	42*	89*
1 d	28*	46*	16†	13†
2 d	19†	15	–9	9
3 d	19	13†	–23	–19
4 d	24*	6	–18†	–24*
Enalapril (20)				
1 h	54*	12†	83*	95*
8 h	9	0	34*	54
1 d	21	–5	16†	–31
2 d	13	–8	12	–51*
Ramipril (5)				
1 h	83*	32†	56*	96*
8 h	52*	–5	13†	66*
1 d	39*	–15	7	–17†
4d	32*	–20†	22*	−70*
Lisinopril (10)				
1 h	62*	22*	67*	96*
8 h	56*	16*	62*	88*
1 d	50*	7	17	15*
4 d	29*	16	–11†	–94*
Fosinopril (25)				
1 h	76*	61*	49*	96*
8 h	63*	31*	32*	81*
1 d	20	–7	5	16*
4 d	12	14	24*	–16*

* $p < .01$; † $p < .05$ vs. vehicle-treated animals.

derivative of lisinopril, as the radioligand for labeling ACE. ACE inhibition was evaluated in specific regions of the heart and great vessels (aorta, pulmonary artery, aortic and pulmonary valves, right and left atrium and ventricle) at 4 and 24 h after treatment. In agreement with the previous studies, zofenopril produced a marked (70%–90%) ACE inhibition in all regions of the heart at 4 h, and this effect persisted after 24 h.

Antihypertensive effects

The effects of single doses of zofenopril (2.2, 6.6 and 22 mg/kg) were evaluated in two kidney-one clip renal hypertensive rats (2K-1C) and SHRs (11). In 2K-1C rats, zofenopril produced a dose-dependent antihypertensive effect of long duration (>17 h). At 6.6 mg/kg zofenopril lowered blood pressure by as much as 70 mmHg (220 to 150 mmHg), while at an equimolar dose captopril lowered blood pressure by only 22 mmHg. In SHRs, zofenopril had dose-dependent effects, lowering of blood pressure by 21–33 mmHg. At the highest dose used, zofenopril lowered blood pressure for at least 17 h. The effects of repeated administration of 22 mg/kg zofenopril b.i.d. were evaluated in SHRs (11). Systolic blood pressure (SBP) fell by 47 mmHg (188 to 141 mmHg) by day 14 of the study. The duration of the effect was longer than 12 h as identical SBP values were recorded at either 1 or 12 h after drug administration.

In a recent hemodynamic study in SHRs (24), zofenopril, given in the diet daily for 6 months, reduced mean arterial pressure from 106 to 84 mmHg. The effect was similar to that of hydralazine, but left ventricular systolic force-time integral (a measure of total ventricular load) and left ventricular weight to body weight ratio were significantly reduced only in zofenopril-treated rats.

Cardioprotective activity

In vitro studies

One study evaluated the effects of ACE inhibitors on coronary circulation of isolated rat hearts (38). Captopril (368 μM) and zofenopril (36 μM) significantly increased coronary flow after 5 min of perfusion; this effect was not accompanied by an increase in 6-keto $PGF_{1\alpha}$ overflow in the coronary effluent. The onset of coronary vasodilator action of ramiprilat (39 μM) was slower; the effect was significant only after 20 min of perfusion and was associated with an increase in 6-keto $PGF_{1\alpha}$ outflow, suggesting a different mechanism of action for ACEIs with and without the sulfhydryl group. The effects of captopril (36–1080 μM) and zofenopril (3.6–36 μM) were concentration-dependent, but the pharmacological efficacy as well as the relative potency of zofenopril was definitely higher. Both zofenoprilat and captopril, but not enalaprilat, potentiated the vasodilator effect of bradykinin on coronary vessels of isolated rat hearts (10,20,37,38,39). The ACE inhibitors are likely to cause coronary vasodilatation by a bradykinin-mediated release of nitric oxide, which can be enhanced in the presence of free sulfhydryl groups. ACE inhibitors containing a free sulfhydryl group may, therefore, potentiate nitrates and reverse tolerance to their therapeutic effects, as suggested also by preliminary clinical evidence (37).

Three separate studies investigated the cardioprotective effects of zofenopril in experimental models of global ischemia/reperfusion. In one study, zofenopril and zofenoprilat

were compared with captopril, enalaprilat, ramiprilat, and fosinoprilat (17). Reperfusion released large amounts of lactic dehydrogenase (LDH), an indicator of cell viability or membrane integrity, decreased cardiac function (product of heart rate times left ventricular pressure), and increased end-diastolic pressure (EDP, a reflection of contracture indicating severe damage). Zofenoprilat (10 μM) and zofenopril (3 μM) improved contractile force and reduced EDP and LDH release during reperfusion. These cardioprotective effects of zofenopril or zofenoprilat were largely concentration-dependent. Enalaprilat or ramiprilat (at concentrations up to 400 μM) and fosinoprilat (at concentration up to 100 μM) had no cardioprotective effects. In contrast, captopril (at approximately 400 μM) significantly improved cardiac function during reperfusion, and reduced EDP and LDH release (Fig. 3).

In another study, zofenopril (at 50 μM) was compared with captopril and fosinopril at the same concentration (20). In control experiments left ventricular developed pressure (dp/dt_{max}) and coronary flow were markedly decreased at the end of the reperfusion period, while the release of creatine kinase (CK), a sensitive indicator of membrane injury, was greatly increased. Zofenopril and captopril improved postischemic left ventricular function, increased coronary flow, and reduced CK release, while fosinopril was ineffective. Moreover, zofenopril and captopril, but not fosinopril, reduced lipid peroxidation and membrane content of nonesterified fatty acids during reperfusion.

Ferrari et al. (15) investigated the effects of zofenopril and captopril (both at 1 μM) on the functional and metabolic damage induced by ischemia and reperfusion in isolated rabbit hearts (Fig. 4). Both drugs had cardioprotective effects (although zofenopril was always considerably more effective), improved recovery of the developed pressure, reduced creatine phosphokinase (CPK), norepinephrine and lactate release, but maintained Ca^{2+} homeostasis and phosphorylation capacities of the mitochondria. Zofenopril, but not

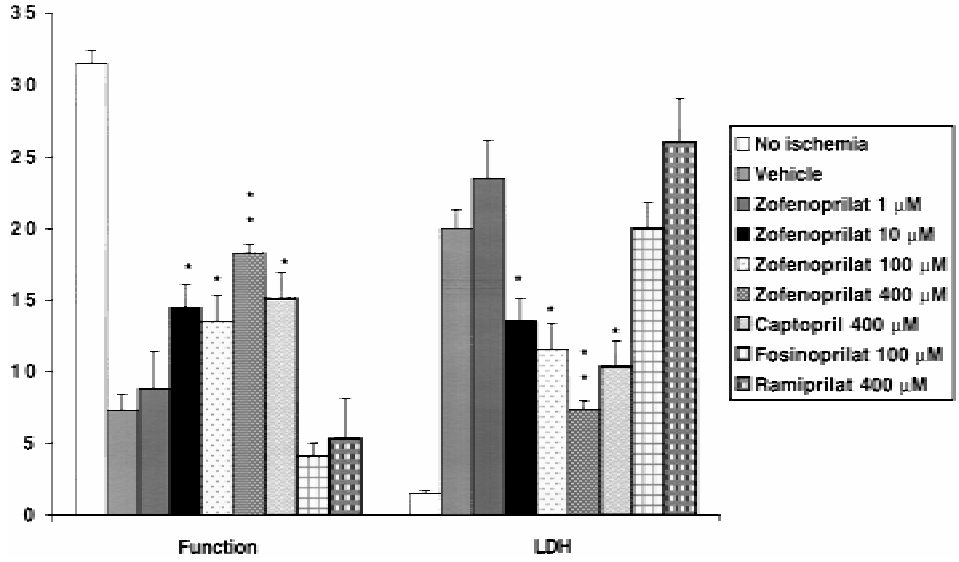


FIG. 3. Effect of zofenoprilat, captopril, fosinoprilat, and ramiprilat on cardiac function (heart rate × ventricular diastolic pressure/1000) and LDH release (U/g) following global ischemia/reperfusion in isolated rat heart. *$p < .05$; **$p < .01$ as compared to vehicle-treated group (modified from ref. 17).

captopril, attenuated oxidative stress during reperfusion. The authors suggested that the cardioprotective effects of zofenopril and captopril are independent of hemodynamic changes or oxygen free radicals but may be related to the reduction of norepinephrine release.

More recently captopril and zofenoprilat were found to enhance left ventricular (LV) relaxation in isolated working guinea pig hearts without significantly altering early systolic LV performance (2); these effects were not observed with either lisinopril or quinaprilat. The effects of captopril and zofenoprilat were attenuated by nitric oxide scavenger, hemoglobin, and the bradykinin-receptor (B_2) antagonist HOE 140. Thus, the presence of a sulfhydryl group appears to be essential for the LV relaxant effect that is possibly mediated by increased activity of the bradykinin–nitric-oxide pathway.

In other recent studies, zofenoprilat (0.01–1 mM) was shown to protect endothelial function; it abolished the proapoptotic effects of doxorubicin, promoted mitosis of bovine coronary venular endothelial cells (CVEC) (5), enhanced concentration-dependently cell survival, and improved vascular-endothelial-growth-factor–induced proliferation of CVECs (kept 5 days in 0.1% serum to mimic a stress condition) (29). Zofenoprilat (1–100 μM) also promoted angiogenesis in porcine coronary arteries, assessed as pseudocapillary formation in three dimentional fibrin gels (5). Zofenoprilat appears to favor proliferation of coronary endothelial cells, leading to angiogenesis by reversing apoptosis.

In vivo studies

The ability of zofenopril to prevent ischemic myocardial damage was studied *in vivo* in a chronic closed-chest pig model of ischemia and reperfusion (36). Pigs were pretreated

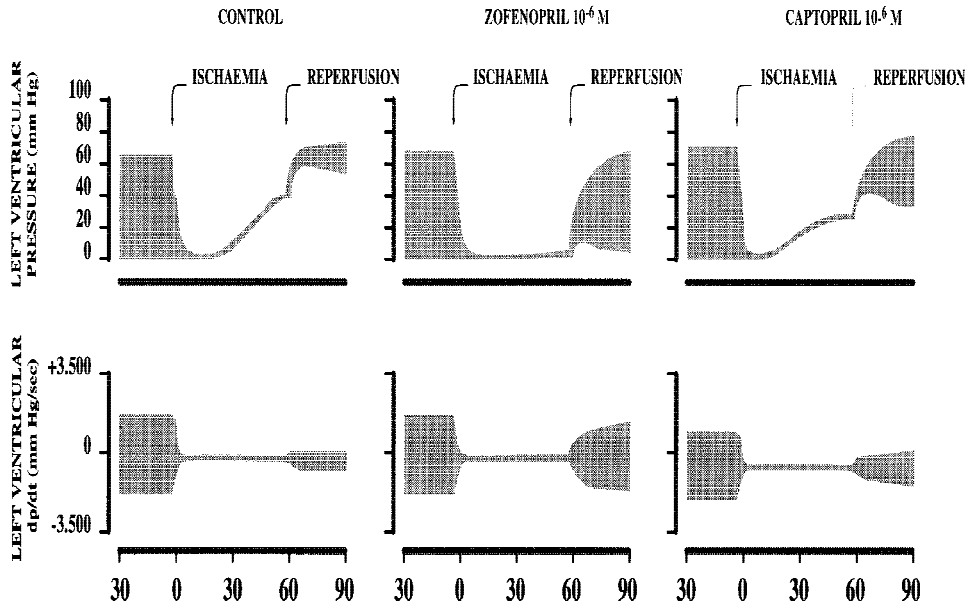


FIG. 4. Typical tracings showing the effect of 10^{-6} M zofenopril and captopril on systolic and diastolic left ventricular (LV) pressure and positive and negative LV(dp/dt) of isolated perfused rabbit heart made temporarily ischemic (60 min) and then reperfused (30 min) (modified from ref. 15).

with zofenopril (approximately 0.5 mg/kg/day p.o.) 2 days prior to ischemia, which was induced by the inflation of a catheter balloon in the left anterior descending coronary artery over 45 min followed by reperfusion. During reperfusion, zofenopril decreased the pressure-rate product and reduced the peak efflux of catecholamines and purine metabolites in the coronary venous effluent. After 2 weeks, signal averaging of the ECG showed the development of late potentials in all untreated animals, whereas in the zofenopril-treated animals late potentials were not observed. Moreover, zofenopril caused a significant reduction of the inducibility of sustained ventricular tachyarrhythmias.

The effects of zofenopril, epicaptopril (the stereoisomer of captopril, which is devoid of ACE inhibitory properties), both at 5 mg/kg i.v., and enalaprilat, 1.5 mg/kg i.v., given at the time of reperfusion on recovery of contractile function after 15 min occlusion of the left anterior descending coronary artery (LAD) were studied in anesthetized open-chest dogs (31). Regional myocardial contractile function (segment shortening) was assessed by sonomicrometry. In the stunned, previously ischemic LAD bed at 3 hours after reperfusion, segment shortening remained depressed, recovering to only −5% of preocclusion baseline. Either of the three drugs attenuated postischemic dysfunction: segment shortening was restored to 33%, 54%, and 83% of baseline value after reperfusion in dogs treated with epicaptopril, zofenopril, or enalaprilat, respectively. These improvements in segment shortening did not appear to be the result of altered oxygen supply or demand after reperfusion, since there was no difference in systemic hemodynamic parameters or myocardial blood flow between the treatment groups. The improved contractile function associated with enalaprilat treatment was largely reversed by indomethacin, which had no effect on the improved contractile function associated with zofenopril, indicating a different mechanism of action for the two drugs.

Two studies evaluated the effects of zofenopril (12–15 mg/kg/day in drinking water) on cardiac remodeling in rats with congestive heart failure after acute myocardial infarction (AMI) due to ligation of the left coronary artery (30,39). In the first study, zofenopril was administered either for 5 days (short-term treatment) or for 6 weeks (long-term–treatment) following AMI. Both groups of animals were evaluated at 6 weeks after the first treatment (30). Experimental AMI increased left ventricular cavity. Both short- and long-term treatment significantly reduced septal wall thickness. Only long-term treatment reduced left ventricular volume in rats with moderate size infarcts; this effect was accompanied by a decrease in heart-weight to body-weight ratio. In the second study, zofenopril or spirapril (2–2.5 mg/kg/day) were administered for 6 weeks. The animals were thereafter sacrificed, and the hearts removed and perfused according to Langendorff (39). In control animals (not treated with ACE inhibitors), ventricles were enlarged and the rate of contractions was decreased. Pretreatment with either zofenopril or spirapril attenuated ventricular enlargement and increased the rate of contractions of isolated hearts.

The effects of zofenopril (10 mg/day for 16 weeks) on cardiac remodeling was evaluated also in dogs with myocardial damage produced by repetitive transmyocardial DC shock (22). In the control group, left ventricular mass and end-diastolic volume, evaluated by magnetic resonance imaging, were significantly increased at the end of the study. Zofenopril suppressed the increase of both ventricular mass and volume, whereas the α_1-adrenoceptor antagonist terazosin or the angiotensin-receptor antagonist losartan failed to attenuate ventricular remodeling (23).

Finally, the cardioprotective effects of zofenopril were evaluated in Golden Syrian hamsters fed a magnesium-deficient diet that is known to produce focal myocardial necrosis and calcification (16). Animals were implanted s.c. with slow-release pellets containing zofenoprilat, captopril, epicaptopril, or enalaprilat at the approximate dose of 1 mg/kg/day. Zofenoprilat, captopril, and epicaptopril reduced both the density and the area of lesions (evaluated by morphometric analysis), while enalaprilat was virtually ineffective.

Mechanism of action

During the last decade, a number of investigators explored the antiischemic/cardioprotective actions of ACE inhibitors. However, the results have been often conflicting and the emerging picture is still not clear. This subject has been reviewed by Przyklenk and Kloner (32), and according to them, some investigators reported protective effects of structurally diverse ACE inhibitors, while others have found them to be ineffective or demonstrated cardioprotective activity only for ACE inhibitors containing sulfhydryl group (SH-ACEIs). The mechanisms responsible for cardioprotection are complex and may be different in various models. In vivo models of cardiac ischemia use mostly coronary occlusion to produce ischemia. The occlusion can be permanent, transient ($=15$ min; models of "stunned myocardium"), or prolonged (models of subendocardial necrosis). We shall not attempt, therefore, a discussion of cardioprotective action by ACE inhibitors in general, and will restrict the discussion to the global ischemia model (isolated rat heart), which was used to study the mechanism of cardioprotective action of zofenopril (15,17,34).

At high concentrations (supramaximal in respect to ACE inhibition in the heart) enalaprilat, ramiprilat, and fosinoprilat were completely devoid of cardioprotective effects, while only zofenoprilat and captopril were effective. This finding ruled out both inhibition of angiotensin II formation and reduced bradykinin breakdown as the mechanism of cardioprotection in the isolated ischemic heart. Enhanced prostaglandin formation was also suggested to be a possible mechanism of cardioprotection. This hypothesis was not supported by findings that in isolated ischemic heart indomethacin only slightly reversed the protective action of zofenoprilat (17). Moreover, in the open-chest dog study reported above (31), indomethacin reversed the cardioprotective effect of enalaprilat but not that of zofenopril.

The potential of SH-ACEIs in scavenging radical oxygen species (ROS) has been proposed as a cocausative factor in the cardioprotection exerted by this class of compounds. This oxygen radical scavenging hypothesis is, however, rather difficult to verify either *in vivo* or *ex vivo* since the antioxidant action is critically affected by the choice of the biological system and by the extent and severity of the oxidative insult. The comparison of the effects of different ACE inhibitors in the open-chest dog model (described above) and the isolated ischemic rat heart is indicative. While in the former zofenopril, captopril, and epicaptopril (the captopril stereoisomer devoid of ACE inhibitory properties) all exerted cardioprotection (31), in the latter the effect of zofenopril was stereoselective: its stereoisomer epizofenopril (devoid of ACE inhibitory properties) had no cardioprotective activity (34). Nevertheless, beneficial cardiovascular effects of SH-ACEIs are well-documented and can often be correlated with the antioxidant activity of these

compounds. Thus, a dual action of ACE inhibition and ROS scavenging has been invoked to explain the protective effect of SH-ACEIs in myocardial dysfunction induced by reperfusion of isolated ischemic rat hearts (34,42). The hydroxyl radical scavenging by zofenopril and captopril (but not by fosinopril) was proposed to be the likely mechanism of cardioprotection from myocardial ischemic-reperfusion injury of isolated rat hearts (20).

In vitro studies in which the photooxidation of riboflavin sensitized by dianisidine was used to generate active oxygen species clearly defined the remarkable difference in the antioxidant action of ACE inhibitors with or without sulfhydryl group (3). Zofenopril, captopril, epicaptopril, and fentiapril were found effective scavengers of nonsuperoxide free radicals, while four nonsulfhydryl-group–containing ACE inhibitors were inactive. Cells and tissues are generally adequately equipped with enzymatic defense systems (i.e., superoxide dismutase, glutathione peroxidase, catalase). These enzymes minimize damage induced by superoxide anions and hydrogen peroxide. The reported ineffectiveness of captopril in scavenging superoxide anions (13,19) suggests limitations in its usefulness as an antioxidant. Although a specific targeting toward the most damaging radical species, such as hydroxyl radicals, could lead to an improved efficiency in the antioxidant defense (7), the antioxidant activity of SH-ACEIs is likely to be therapeutically useful. The scavenging of active oxygen species like singlet oxygen, hydrogen peroxide, and peroxyl radical is relevant in tissue injury. Also SH-ACEIs were reported to reduce the production of superoxide anion induced in neutrophils by zymosan (7).

The protective effects of SH-ACEIs from free radical-induced cell damage have been also assessed in cultured endothelial cells exposed to a superoxide anion and hydroxyl radicals generating system (21). Preincubation of the cells with captopril, epicaptopril, or zofenopril produced a concentration-dependent (10–200 μM) inhibition of malonyldialdehyde formation. Both loss of cell viability and membrane blebbing were reduced by SH-ACEIs at concentrations as low as 10 μM. In contrast, lisinopril and enalaprilat at concentrations up to 200 μM were ineffective. Further experiments on the effects SH-ACEIs on hydroxyl radical formation using ESR spin-trapping techniques indicate that their mechanism of protection of endothelial cells from lipid peroxidation-induced damage may involve scavenging of hydroxyl radicals.

More recently (12), zofenoprilat was shown to prevent irreversible inactivation of purified bovine aldose reductase (ALR2) by 4-hydroxy-2-nonenal (HNE). HNE is a major aldehydic product of lipid peroxidation, perhaps the most reactive one, which induces several harmful actions in biological systems. ALR2 is likely to be the major detoxification pathway which prevents the cellular damage by HNE. Zofenoprilat is capable of reversibly modifying ALR2 by means of the same thiolating action as exerted by glutathione. However, at variance with glutathione that reduces the enzymatic activity of ALR2, zofenoprilat does not affect its activity. These results suggest that zofenoprilat, by maintaining an active ALR2, is capable of preserving or enhancing the ability of cells to detoxify ROS (12).

Finally, another mechanism of action has recently been proposed for zofenopril (34). The effects of zofenopril were reversed by two structurally different blockers of the ATP-sensitive potassium channel (K_{ATP}): glyburide and 5-hydroxydecanoate. Isobolographic analysis demonstrated that treatment with a combination of zofenopril and cromakalim (a K_{ATP} opener) resulted in superadditive response in the ischemic myocardium,

and K_B analysis demonstrated that glyburide is a noncompetitive antagonist in the presence of zofenopril and a competitive antagonist in the presence of cromakalim. The results suggest a link between the cardioprotective effects of zofenopril and the K_{ATP} channel. This activity appears to be a receptor-mediated event, involving a mechanism different from that of the classical K_{ATP} openers, such as cromakalim.

It was shown more recently that zofenopril, at relatively high concentrations, is capable of relaxing guinea pig thoracic aorta and bovine coronary artery precontracted with various agonists. This relaxation was unaffected by glyburide in bovine coronary artery and only slightly attenuated in guinea pig aorta, indicating that opening of the K_{ATP} channels is not a major determinant of zofenopril-induced vasodilation in these vascular preparations (18).

TOXICOLOGY

To evaluate safety of zofenopril extensive, toxicological studies were conducted with this drug (unpublished, data on file at Menarini Ricerche S.p.A., Firenze, Italy). The mean lethal single oral dose of zofenopril in mice or rats was higher than 8 g/kg, and in dogs it was higher than 1.6 g/kg. Subchronic and chronic oral toxicity studies (4 weeks and 6–12 months) of zofenopril were carried out in rats, dogs, and cynomolgus monkeys. The reproductive toxicity studies included a rat fertility study, evaluation of general reproductive performance, teratology studies in rats and rabbits, and a rat peri- and postnatal studies.

Most changes found during the course of the general toxicological studies of zofenopril are common for ACE inhibitors. Many of these changes are due to exaggerated pharmacological effects of the drug. These changes include a decrease in erythrocytic parameters, an increase in serum urea nitrogen, a decrease in heart weight, and hyperplasia of the juxtaglomerular cells. Also the maternotoxic and fetotoxic effects observed in the rabbit segment II reproduction study are typical of ACE inhibitors and are well documented in the literature (14,27,41).

The mutagenic potential of zofenopril was evaluated in a vast array of in vitro and in vivo tests, which demonstrated the absence of mutagenic or clastogenic activities of the drug. Carcinogenicity studies were carried out in mice and rats. Zofenopril had no carcinogenic effects.

PHARMACOKINETICS

Numerous absorption, distribution, metabolism, and excretion (ADME) studies were carried out in rats, dogs and cynomolgous monkeys with [^{14}C]zofenopril (unpublished data on file at Menarini Ricerche S.p.A., Firenze, Italy).

Absorption

Based on urinary excretion after oral and i.v. administration, the oral absorption of zofenopril was estimated to exceed 80% in rats and dogs and 70% in monkeys. The bioavailability of zofenoprilat after an oral dose of zofenopril was 100% in rats, >70% in dogs, and 50% in monkeys. Based on a comparison of AUC values after oral and i.v. administration, the oral absorption of [^{14}C]zofenopril in dogs was 100% and the bioavail-

ability of zofenoprilat was 93%. Following an oral administration of zofenopril, t_{max} values for zofenoprilat were between 0.3–0.9 h and $t_{1/2}$ values between 5–7 h for either of the three animal species.

Based on the recovery of radioactivity after administration of [^{14}C]zofenopril to rats, minimal absorption rats averaged 54% from stomach, 57% from duodenum, 70% from jejunum, 46% from ileum, and 35% from colon (26). In dogs the minimal absorption rate from the colon averaged 11%.

The relative contribution of gut, liver, and lungs as sites of first-pass bioactivation of zofenopril to zofenoprilat was evaluated in dogs receiving zofenopril by intraarterial, intravenous, intraportal, or oral routes. Gut and liver had a high intrinsic capacity to hydrolyze zofenopril, while that of the lungs is low. Overall, 95% of the orally administered dose of zofenopril was hydrolyzed during the first pass. Because the prodrug is sequentially exposed to the gut, liver, and lungs, the contribution of the gut to the overall first-pass hydrolysis (about 87%) was estimated to be higher than that of the liver (<10%) or lungs (<2%) (25).

Distribution

Following a single oral dose of [^{14}C]zofenopril, the highest concentration of total radioactivity was found at 0.5 h after treatment in the organs involved in absorptive/excretory processes as well as in the heart and vasculature. Thereafter, the radioactivity levels fell in most organs but remained relatively constant in heart and vasculature. After 24 h, the radioactivity levels in the heart and vasculature were higher than in plasma (Fig. 5).

FIG. 5. Time course of distribution of zofenoprilat in plasma, heart, and aorta after a single oral administration of [^{14}C]zofenopril (40 mg/kg) in rats.

Metabolism

A very low amount of unchanged zofenopril was found in blood and urine of rats, dogs, and monkeys. In rats, zofenoprilat was the major metabolite in the blood, whereas five radioactive compounds were found in the urine. Also, zofenoprilat was one of the major radioactive metabolites in blood in dogs, while only small amounts of zofenoprilat were detected in urine; numerous radioactive metabolites, including disulfide dimer of zofenoprilat, were present in the rat urine. Biotransformation profiles of zofenopril in urine and blood extracts from rats, dogs, and monkeys given an oral dose of zofenopril or an intravenous dose of zofenoprilat were compared with those of humans and found qualitatively similar. In each of the four species zofenoprilat was the major metabolite. Several purified metabolites were isolated from urine samples collected from human subjects after administration of [^{14}C]zofenopril or zofenoprilat. Eight metabolites accounting for about 76% of the urinary radioactivity were identified. The results indicated that zofenopril is extensively hydrolyzed to zofenoprilat, which is then metabolized mainly by conjugation pathways. Enzymatic oxidation in the 4-phenylthio position also occurred (Fig. 6).

Excretion

After oral administration of [^{14}C]zofenopril to bile-cannulated rats, about 18% of the dose was recovered in bile, 67% in urine, and 7% in the gastrointestinal tract and feces. After i.v. administration of zofenoprilat to rats, the radioactivity was excreted primarily in the urine and less in the feces. These results indicate preferential urinary excretion accompanied by a substantial biliary excretion. A similar excretory pattern was found in dogs and monkeys.

CONCLUSIONS

ACE inhibitors represent one of the major advances in cardiovascular therapy over the past 20 years. Their widespread clinical use could not have been anticipated even by their most enthusiastic advocates. Although a novel class of agents acting on the renin-angiotensin system, namely the AT-receptor antagonists, were recently introduced, ACE inhibitors are likely to remain one of the most widely used classes of cardiovascular agents for many years.

Many different ACE inhibitors have been marketed. They differ from each other in regard to their chemical structure, potency, tissue affinity, pharmacokinetics etc. One of the tasks for the future research is to compare ACE inhibitors pharmacologically and clinically and to determine whether in selected indications some of them may be preferable to others.

Zofenopril is the most recent ACE inhibitor introduced into therapy. Its high lipophilicity is one of its main characteristics. The lipophilicity determines various biological properties, such as oral absorption, an appreciable degree of biliary excretion and probably more importantly, an enhanced tissue penetration. In animal models, orally administered zofenopril is unique in producing a long-lasting inhibition of heart tissue ACE. This property is probably determined by high efficiency with which this prodrug is taken by the heart tissue and promptly hydrolyzed to the active inhibitor by cardiac esterases. Angio-

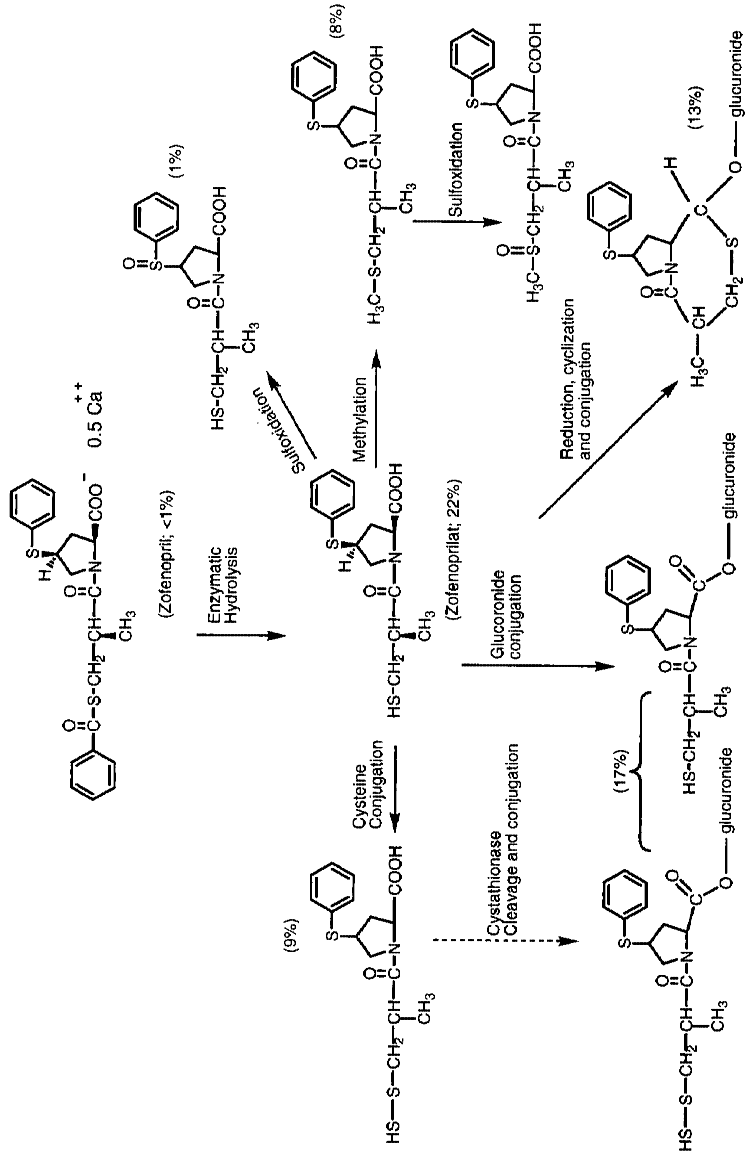


FIG. 6. Proposed biotransformation pathways for zofenopril calcium and zofenoprilat in humans. The percent of radioactivity excreted in urine are shown in parentheses.

tensin II has a wide range of effects that have been implicated in the pathophysiology of cardiac hypertrophy and heart failure. In addition to its vasopressor effect, angiotensin II promotes deleterious hypertrophy and fibrosis as a growth factor for cardiocytes and fibroblasts. Since angiotensin II has also arrhytmogenic and cardiotoxic effects probably due to enhanced release of norepinephrine from cardiac sympathetic nerves, the importance of reducing formation of angiotensin II in the heart is obvious. Moreover, zofenopril, like captopril but unlike most other ACE inhibitors, contains a sulfhydryl moiety, which is capable of scavenging oxygen free radicals. This property is likely to determine some pharmacological properties of zofenopril that are not shared with many other ACE inhibitors. These properties include an increase in coronary blood flow, reversal of nitrates tolerance, *in vitro* and *in vivo* antiischemic effects, and enhanced left ventricular relaxation. Even more attractive are the recent preliminary data regarding the protective effects of zofenopril in endothelial cells and promotion of coronary angiogenesis, an observation that has to be confirmed in other experimental models. Special clinical studies are being designed to evaluate whether any of the unusual beneficial effects of zofenopril can be translated into a clinically significant improvement over the existing ACE inhibitors.

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