FEBS Letters 361 (1995) 22–24 FEBS 15209

Superoxide dismutase and glutathione peroxidase activities are increased by enalapril and captopril in mouse liver

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Received 4 February 1995

Abstract We have characterized the effect of angiotensin converting enzyme (ACE) inhibitors on the activity of CuZn-superoxide dismutase (CuZn-SOD), Mn-superoxide dismutase (Mn-SOD), catalase, and selenium-dependent glutathione peroxidase (Se-GPx). CF1 mice (4-month-old females) were administered water containing enalapril (20 mg/l) or captopril (50 mg/l), during 4 to 11 weeks. After 11 weeks, enalapril treatment caused an increase in the activity of CuZn-SOD, Mn-SOD and Se-GPx, from 19 ± 4 to 46 ± 7, 2.1 ± 0.2 to 3.8 ± 0.2 units/mg protein and 27 ± 3 to 54 ± 3 milliunits/mg protein, respectively. After 11 weeks, captopril treatment increased the activities ($P < 0.05$) of CuZn-SOD, MnSOD and Se-GPx to 35 ± 4, 2.9 ± 0.2 units/mg protein, and 38 ± 2 milliunits/mg protein, respectively. Catalase activity was not affected by the treatments. These results suggest that ACE inhibitors may protect cell components from oxidative damage by increasing the enzymatic antioxidant defenses.

Key words: Oxygen radical; Enalapril; Captopril; Antioxidant; Aging; Hypertension

1. Introduction

Superoxide dismutases (SOD), catalase and glutathione peroxidases (GPx) are the main antioxidant enzymes found in aerobic organisms, and they constitute part of the physiological defenses against oxidative stress [1]. Several evidences indicate that an adequate balance among the antioxidant enzymes is necessary to minimize the toxic effects of reactive oxygen species (ROS) [2–4]. Moreover, it was recently shown that the simultaneous induction of several antioxidant defenses, enzymatic and non-enzymatic, is necessary to increase the mean life-span of frogs [5].

Angiotensin converting enzyme (ACE) inhibitors, frequently used in the treatment of arterial hypertension and cardiac failure, have been shown to possess other pharmacological effects. They have been shown to decrease myocardial sclerosis in different pathological situations [6,7], attenuate post-ischemic myocardial dysfunction [8,9], reduce glomerulosclerosis in diverse experimental models [10, 11], and diminish age-related renal interstitial fibrosis [12]. The mechanisms underlying those observations are not well understood. They may include hemodynamic effects, and/or the stimulation of the synthesis of cytoprotective prostaglandins [8,9]. A free radical scavenger action has also been postulated [13–15].

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In previous work, we found that mice chronically treated with enalapril, a non-sulfhydryl containing ACE inhibitor, showed a reduction in myocardial and glomerular sclerosis and an increase in the number of mitochondria in myocardiocytes and hepatocytes, which was correlated with an increase in their survival, when compared with untreated controls [16]. Considering that it has been widely postulated that ROS are causally involved in the aging process, we hypothesized that enalapril might have altered the prooxidant/antioxidant balance, in favor of the latter, protecting cells from oxidative damage. The underlying mechanisms may comprise either free radical scavenger properties of enalapril per se, or enalapril-mediated enhancement of antioxidant defenses. Efforts to demonstrate the potential ability of this ACE inhibitor to trap free radicals have generated conflicting results [17–22]. To study the possibility that the effect of enalapril could rely upon its ability to enhance antioxidant enzymatic defenses, we measured the activities of CuZn-SOD, Mn-SOD, catalase, and Se-dependent glutathione peroxidase (Se-GPx) in the liver of mice chronically treated with enalapril or captopril. An increased activity of both CuZn-SOD, Mn-SOD and of Se-GPx was observed in the liver of mice treated during 11 weeks either with captopril or enalapril.

2. Materials and methods

All reagents, enzymes and enzyme substrates were reagent grade and were obtained from Sigma Chemical Company (Saint Louis, MO).

Female CF-1 mice, 4 months old, were randomly separated into 3 groups that were administered either tap water (controls) or water containing 20 mg/l enalapril maleate (Merck, Sharp & Dohme, West Point, PA), or 50 mg/l captopril (Bristol-Myers Squibb, Princeton, NJ), for 4–11 weeks. The doses were chosen according to Ferder et al. [16]. Mice received captopril at a higher dose than enalapril due to the shorter half-life of that compound. Animals had free access to a Purina type I diet (Cargill, Buenos Aires, Argentina). Water consumption was determined daily. Body weight and blood pressure were determined by tail plethysmography 1 or 2 days before killing.

At the end of each treatment period (4, 7, 9, and 11 weeks), mice were anesthetized with chloroform and the livers were excised after perfusion with 150 mM NaCl at 4°C. For enzymatic determinations, the livers were homogenized in 10 vol of 120 mM KCl, 30 mM potassium phosphate, pH 7.4, and centrifuged at 600 × *g* for 10 min. Enzyme activities were measured in the supernatant. Protein content was determined using bovine serum albumin as standard [23].

Total SOD activity was determined following spectrophotometrically the inhibition of cytochrome *c* reduction by superoxide anion at 550 nm [24]. One unit of SOD was defined as the amount of enzyme necessary to cause a 50% of inhibition of the reduction of cytochrome c (20 μM) by superoxide anion generated by the xanthine (50 μM)/xanthine oxidase (5 nM) system. To determine Mn-SOD activity, CuZn-SOD was wholly inhibited (100%) with 1 mM NaCN. CuZn-SOD activity was calculated by subtracting Mn-SOD from total SOD activity. Catalase activity, was determined by measuring the decrease in the absorbance at 240 nm in a reaction medium containing 50 mM potassium phos-

SSDI 0014-5793(95)00137-9

phate buffer, pH 7.0, and 10 mM H_2O_2, and calculating the reaction constant of pseudo-first order (k') from the decrease in the absorbance of H_2O_2 (E_{240} = 40 $M^{-1} \cdot cm^{-1}$). Catalase content was expressed as pmol catalase/mg of protein, using $k = 4.6 \times 10^7$ $M^{-1} \cdot s^{-1}$ [1]. Se-GPx was determined according to Günzler [25] with modifications, by following NADPH oxidation at 340 nm. Homogenate samples were incubated in the presence of 5 mM GSH, 0.5 mM NADPH, 1 mM NaN_3, 0.125 U/ml glutathione reductase, and 0.5 mM H_2O_2, in 50 mM potassium phosphate, pH 7.7. One unit Se-GPx was defined as the amount of enzyme that oxidized one μmol of NADPH/min at room temperature.

Statistical analyses were performed using the routines available in Statview SE + Graphics v 1.03 (Abacus Concepts, Inc., Berkeley, CA).

3. Results

A non-significant increase in body weight was observed in the mice treated with either enalapril or captopril as compared to controls. At the beginning of treatment, body weights were 14.6 ± 1.3, 15.4 ± 1.2, 14.0 ± 1.4 g, for control, enalapril- and captopril-treated mice, respectively. After 11 weeks of treatment, the corresponding values were 18.2 ± 1.8, 21.3 ± 1.5 and 21.5 ± 1.4 g. Similar water intake (16 ± 2 ml/d) was observed for the three experimental groups throughout the study. Enalapril and captopril had no effect on either liver weight (2.8 ± 0.2 g), liver protein content (117 ± 5 mg/g liver) or mice blood pressure after 11 weeks of treatment (systolic: 112 ± 1.3 mmHg, n = 7; diastolic: 81 ± 1 mmHg, n = 7, at week 11).

The activities of liver CuZn-SOD, Mn-SOD, Se-GPx, and catalase in the control mice were not modified throughout the treatment period (4 to 11 weeks), (Tables 1–3). Enalapril increased the activity of CuZn-SOD, Mn-SOD, and Se-GPx after 9 weeks of treatment, with a maximal effect at week 11. At week 11 the activities of CuZn-SOD, Mn-SOD, and Se-GPx in the livers from enalapril-treated mice were higher (142, 81, and 100%, respectively) compared to controls (Tables 1–3). Captopril also increased the activity of the antioxidant enzymes, but the effect was observed earlier during treatment. At week 4 the activities of liver CuZn-SOD and Se-GPx were significantly increased (48 and 61%, respectively) compared to controls. The activity of these enzymes remained unchanged until the end of the treatment (11 weeks). A significant effect of captopril on Mn-SOD was only noticeable at week 11, reaching an increase of 38% over the control value ($P < 0.05$) (Table 2).

To characterize the possible effect of acute captopril treatment on the antioxidant enzyme activities, a group of six mice was treated during two days with the ACE inhibitor. The activities of liver CuZn-SOD, Mn-SOD, and Se-GPx from the treated mice were not different than control values (data not shown).

Table 1
CuZn-superoxide dismutase activity in liver from mice treated with enalapril or captopril

Treatment	CuZn-SOD activity (units/mg protein) Time of treatment (weeks)			
	4	7	9	11
Control	21 ± 2 (6)	21 ± 2 (6)	25 ± 2 (3)	19 ± 4 (6)
Enalapril	24 ± 2 (6)	24 ± 2 (6)	43 ± 5 (3)[a,b]	46 ± 7 (5)[a,b]
Captopril	31 ± 2 (6)[b]	36 ± 3 (7)[b]	n.d.	35 ± 4 (6)[b]

[a]Statistically different ($P < 0.05$) from 4 weeks-group under same treatment; [b]statistically different ($P < 0.05$) from control group at the same week of treatment; n.d., not determined. Data are presented as mean ± S.E.M. The number of animals is shown in parenthesis.

Table 2
Mn-superoxide dismutase activity in liver from mice treated with enalapril or captopril

Treatment	Mn-SOD activity (units/mg protein) Time of treatment (weeks)			
	4	7	9	11
Control	2.1 ± 0.2 (6)	2.3 ± 0.3 (6)	2.2 ± 0.4 (3)	2.1 ± 0.2 (6)
Enalapril	2.6 ± 0.4 (6)	2.1 ± 0.3 (6)	3.1 ± 0.3 (3)[a,b]	3.8 ± 0.2 (5)[a,b]
Captopril	2.6 ± 0.2 (6)	2.3 ± 0.1 (6)	n.d.	2.9 ± 0.2 (6)[a,b]

[a]Statiscally different ($P < 0.05$) from 4 weeks-group under same treatment; [b]statistically different ($P < 0.05$) from control group at the same week of treatment; n.d., not determined. Data are presented as mean ± S.E.M. The number of animals is shown in parenthesis.

Catalase activity did not vary in the livers of either control, enalapril- or captopril-treated mice during the treatment period (4–11 weeks). Values were 12.4 ± 3.1 (n = 7), 10.8 ± 0.4 (n = 5) and 10.7 ± 1.0.(n = 6) pmol catalase/mg protein, for control, enalapril-, and captopril-treated mice respectively, at 4 weeks of treatment. After 11 weeks of treatment, the corresponding values were 11.8 ± 0.3, 10.2 ± 1.7 and 10.7 ± 0.9 pmol catalase/mg protein.

4. Discussion

We have previously shown [16], that the reduction in the number of mitochondria that occurs with age in mouse hepatocytes and miocardiocytes was prevented by chronic treatment with enalapril. In this paper, we show that enalapril and captopril increase the activities of CuZn-SOD, Mn-SOD, and Se-GPx in mouse liver. This increase in antioxidant defenses should protect mitochondrial components from oxidative damage, preventing the decreased mitochondria number that occurs with age.

Antioxidant enzymes have been reported to increase in eukaryotic cells by treatments with X- [26] and UV-radiation [27], visible light [28], and paraquat [29]. In mammals Mn-SOD is induced by diverse agents, whereas CuZn-SOD is a weakly inducible enzyme [30]. The protective role of increasing the antioxidant defenses was reported in several systems. In a model of cardiac ischemia-reperfusion the augmentation of the activities of total SOD and Se-GPx protects from reactive oxygen species-mediated injury [31]. SOD and catalase exogenously administered, reduce the cellular lesions caused by myocardial ischemia-reperfusion in dogs [32]. López-Torres et al. found that enhanced levels of CuZn-SOD, glutathione reductase, glutathione and ascorbate, are correlated with higher survival of catalase-inhibited frogs [5]. It has been recently reported that the simultaneous overexpression of CuZn-SOD and catalase diminishes oxidative stress and increases maximum life-span in *Drosophila melanogaster* [33]. The increase in the activities of the antioxidant enzymes may be one of the mechanisms responsible for the protective effects shown by ACE inhibitors *in vivo* in different pathologies [6–12].

Interestingly, enalapril and captopril treatments increased both the activities of SOD and Se-GPx, but the activity of catalase remained unchanged. The coordinate action of SOD, catalase and/or GPx appear to be necessary to avoid the formation of the highly damaging hydroxyl radical [3,4]. Se-GPx is localized in the cytoplasm and in the mitochondria. Thus, the

Table 3
Glutathione peroxidase activity in liver from mice treated with enalapril or captopril

Treatment	Se-GPx activity (milliunits/mg protein) Time of treatment (weeks)			
	4	7	9	11
Control	28 ± 2 (6)	34 ± 2 (6)	36 ± 4 (2)	27 ± 3 (7)
Enalapril	32 ± 3 (6)	26 ± 4 (6)	58 ± 3 (3)[a,b]	54 ± 3 (5)[a,b]
Captopril	45 ± 3 (6)[b]	36 ± 5 (6)	n.d.	38 ± 2 (6)[b]

[a]Statistically different ($P < 0.05$) from 4 weeks-group under same treatment; [b]statistically different ($P < 0.05$) from control group at the same week of treatment; n.d., not determined. Data are presented as mean ± S.E.M. The number of animals is shown in parenthesis.

observed increase in Se-GPx activity could counteract for the increased production of H_2O_2 that might be produced by the rise in the activities of cytosolic CuZn-SOD and mitochondrial Mn-SOD.

The mechanism(s) by which the ACE inhibitors could increase the antioxidant enzymes activities are unknown. The doses of enalapril or captopril used in this study did not modify arterial pressure. Consequently, it is improbable that variations in arterial pressure or in organ hemodynamics play a role in the present results. Moreover, considering that the liver does not usually respond to changes in arterial pressure or undergo hemodynamic variations, it is more likely that the increase in the activities of the antioxidant enzymes could be due to: (a) a direct effect of the ACE inhibitors on enzyme synthesis or activity; (b) a secondary effect resulting from the consequences of ACE metabolic actions: inhibition of angiotensin II synthesis, inhibition of aldosterone formation and release, stimulation of renin production, increased cellular sensitivity to catecholamines, potentiation of bradykinins, etc. The present study does not allow to underscore any of these possibilities. Recently, low molecular weight peptides which modify plasma and erythrocyte SOD activity were isolated from human plasma [32]. Considering that enalapril and captopril are peptide analogs, they could modify the activities of SOD and Se-GPx through a mechanism similar to that of the regulatory low molecular weight peptides found in plasma.

The present results suggests that by increasing enzymatic antioxidant defenses, ACE inhibitors may protect cell components from ROS-mediated damage. This effect could afford a biochemical mechanism to explain the ACE inhibitors beneficial actions observed in several degenerative diseases.

Acknowledgments: This work was supported with grants from the University of Buenos Aires and Antorchas Foundation to César G. Fraga and from the Institute of Nephrology, Jewish Hospital. CGF is an investigator from CONICET.

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