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The effect of angiotensin-converting enzyme inhibition on endothelial function and oxidant stress

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Abstract

Angiotensin-converting enzyme (ACE) inhibitors effectively interfere with the renin–angiotensin system and exert various beneficial actions on vascular structure and function beyond their blood pressure-lowering effects. Data from experimental studies showed that angiotensin-converting enzyme inhibitors can attenuate the development of atherosclerosis in a wide range of species. The postulated mechanisms of this atheroprotective effect are the antioxidant actions of angiotensin-converting enzyme inhibitors and their enhancement of the endothelial elaboration of bioactive nitric oxide. The aim of this study was to assess the comparative effects of three angiotensin-converting enzyme inhibitors on endothelial nitric oxide production and action, and on endothelial oxidative stress. Using bovine aortic endothelial cells in culture grown to confluence, we examined the effects of 1, 10, 30 and 60 μM of each of captopril, zofenopril and enalapril on nitrite/nitrate accumulation in the media, cyclic GMP accumulation in the cell lysate, and F_2-isoprostanes in lipid extracts from the cells. Results showed that the sulfhydryl angiotensin-converting enzyme inhibitor zofenopril has unique properties compared with captopril and enalapril. This compound improves nitric oxide production and bioactivity, and does so in conjunction with decreased endothelial cell oxidant stress. The biochemical basis for this protective mechanism is not entirely clear; however, these actions suggest that zofenopril may reduce endothelial effects of risk factors for atherothrombotic disease.

Keywords: Angiotensin-converting enzyme; Endothelium; Nitric oxide (NO); Oxidative stress

1. Introduction

Angiotensin-converting enzyme (ACE) inhibitors interfere with the renin–angiotensin system and reduce the risk of myocardial infarction, stroke and cardiovascular death exerting various beneficial actions on cardiac and vascular structures beyond their blood pressure-lowering effects (Lonn, 2002). Indeed, controlled clinical trials have shown that angiotensin-converting enzyme inhibitors improve endothelial function, and cardiac and vascular remodeling. Angiotensin-converting enzyme inhibitors may reduce atherogenesis in experimental models through various protective mechanisms (Hayek et al., 1998, 1999; Napoli et al., 1999; Keidar et al., 2000; de Nigris et al., 2001; Chobanian et al., 1990; Aberg and Ferrer, 1990; Rolland et al., 1991; Charpiot et al., 1993; Kowala et al., 1994), including antiproliferative effects on vascular smooth muscle cells (Daemen et al., 1991; Li et al., 1999), reduction of blood pressure (Chobanian et al., 1990; Aberg and Ferrer, 1990) and low-density lipoprotein (LDL) oxidation (Hayek et al., 1995, 1998, 1999; Napoli et al., 1999; Keidar et al., 2000; de Nigris et al., 2001), inhibitory effects on platelet activation (Napoli et al., 1999; de Nigris et al., 2001; Keidar et al., 1996), modulation of proinflammatory signals in the vasculature (Gonzalez et al., 2000), reduction of macrophage accumulation (Napoli et al., 1999; Keidar et al., 2000; de Nigris et al., 2001; Kowala et al., 1994), and improvement of endothelial dysfunction (Rolland et al., 1991; Buikema et al., 2000).

Endothelial nitric oxide production is one manifestation of normal endothelial function. Angiotensin-converting enzyme inhibitors, in part, promote endothelial function by their antioxidant effects and by enhancing bradykinin-mediated endothelial nitric oxide synthesis. Thus, the goal of the present study was to assess in vitro the comparative

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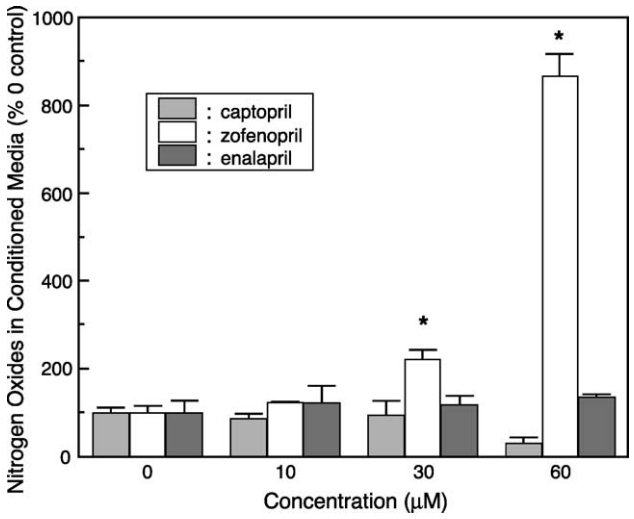

Fig. 1. Effect of different angiotensin-converting enzyme inhibitors on nitrogen oxides. Nitrite and nitrate were measured in the conditioned media of bovine aortic endothelial cells stimulated with A23187, as described in Section 2. Baseline (100%) values were 1.10 ± 0.10 pmol/mg cell protein. Each bar represents the average of three experiments each performed in duplicate. *$P < 0.001$.

effects of three different angiotensin-converting enzyme inhibitors on endothelial nitric oxide production and action, and on endothelial oxidative stress.

2. Materials and methods

2.1. Drugs

Zofenopril, a sulfhydryl angiotensin-converting enzyme inhibitor derivative of the amino acid proline ([1(*S*),4(*S*)]-1(3-mercapto-2-methyl-1-oxopropyl)4-phenylthiol-L-proline-*S*-benzothioester), has been shown to have beneficial cardiovascular effects in patients with myocardial infarction or heart failure (Ambriosini et al., 1995; Borghi et al., 1999) and antiatherogenic activity (Napoli et al., 1999; de Nigris et al., 2001). Thus, it was compared with two other angiotensin-converting enzyme inhibitors, one sulfhydryl-containing, captopril, and the other non-sulfhydryl-containing, enalapril. All angiotensin-converting enzyme inhibitors were kindly provided by Dr. Stefano Evangelista (Milan, Italy). The molecular weight of zofenopril is 448.59 g/mol and its lipophilicity is higher than that of captopril (3.5 vs. 0.004 octanol–water distribution coefficient at pH 7.0) (DeForrest et al., 1989).

2.2. Cell culture

Bovine aortic endothelial cells (Cell Systems) were grown to confluence in Dulbecco's modified Eagle's medium that was supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 10 μg/ml streptomycin. On average, cells were passaged, twice weekly; harvesting was performed using 0.5% trypsin/EDTA. Experiments were conducted on cells from passages 4 to 12. Cells grown to confluence were used to examine the effect of 0, 1, 10, 30, and 60 μM of captopril, zofenopril, and enalapril incubated for 1 h on nitrite/nitrate accumulation in the media, cyclic GMP accumulation in the cell lysate, and F_2-isoprostanes in lipid extracts from the cells following stimulation for 5 min with 5 μM A23187. Each experiment was conducted three times in duplicate.

2.3. Cyclic GMP determination

Confluent bovine aortic endothelial cells were pretreated with 0.5 μM isobutylmethylxanthine for 15 min at 37 °C. The cells were then stimulated with 5 μM A23187 for 10 min in the presence of isobutylmethylxanthine. Six percent ice-cold trichloroacetic acid was added to stop the reaction, and the cells were then subjected to three freeze–thaw cycles. Cell supernatants were extracted with water-saturated ether, dried under nitrogen gas at room temperature, then acetylated with acetic anhydride. Cyclic GMP levels were determined using a cyclic GMP enzyme-linked immunoassay (Cayman Chemical).

2.4. Nitrite/nitrate determination

Concentrations of nitrite and nitrate in the conditioned media of bovine aortic endothelial cells were determined using a fluorimetric method (Cayman Chemical).

2.5. Isoprostane determinations

The F_2-isoprostane $IPF_{2\alpha}$-III was measured using a commercially available enzyme-linked immunoassay (Cay-

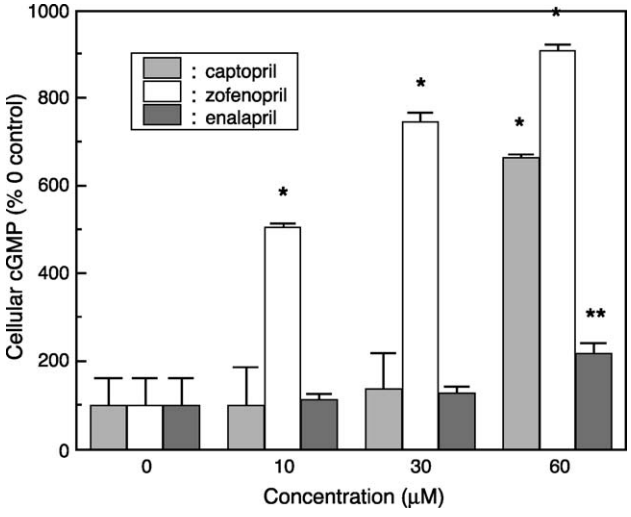

Fig. 2. Effect of different angiotensin-converting enzyme inhibitors on cyclic GMP levels. Cyclic GMP (cGMP) was measured in cellular extracts of bovine aortic endothelial cells stimulated with A23187, as described in Section 2. Baseline (100%) values were 126 ± 5 pmol/mg cell protein. Each bar represents the average of three experiments each performed in duplicate. *$P < 0.001$, **$P < 0.01$.

man Chemical). The isoprostanes were extracted from the bovine aortic endothelial cells as previously described (Forgione et al., 2002).

2.6. Statistical analysis

Data are expressed as mean ± standard error of the mean. Comparison among groups was performed using analysis of variance with post-hoc Newman–Keuls analysis. *P*-values < 0.05 were considered statistically significant.

3. Results

Nitrite/nitrate accumulation was used as a measure of nitric oxide production by bovine aortic endothelial cells. As shown in Fig. 1, only zofenopril enhanced nitrite/nitrate production (given as nitrogen oxides) significantly over the full range of concentrations used ($P < 0.01$). This increase in nitrogen oxides was associated with an increase in nitric oxide bioactivity as demonstrated by the significant increase in endothelial cell cyclic GMP accumulation in response to A23187 ($P < 0.01$), as shown in Fig. 2. Again, this effect was notable only for zofenopril over the range of concentrations used; captopril had an effect on cyclic GMP accumulation, but only at the highest concentration tested.

The effect of these agents on the marker of oxidant stress, F_2-isoprostanes, is shown in Fig. 3. Here, both zofenopril ($P < 0.01$) and captopril ($P < 0.05$) were effective in reducing the levels of this marker over the range of concentrations tested with zofenopril showing a trend toward greater potency in this action than captopril. The reduction in F_2-isoprostanes

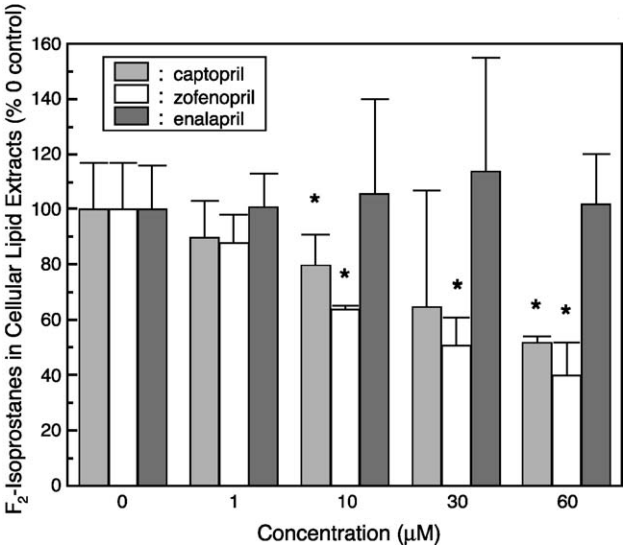

Fig. 3. Effect of different angiotensin-converting enzyme inhibitors on F_2-isoprostanes. $IPF_{2\alpha}$-III was measured in lipid extracts of bovine aortic endothelial cells stimulated with A23187, as described in Section 2. Baseline (100%) values were 45 ± 3 pmol/mg cell protein. Each bar represents the average of three experiments each performed in duplicate. *$P < 0.02$.

correlates with the increase in nitrite/nitrate ($R = 0.92$, $P = 0.01$) and in cyclic GMP ($R = 0.94$, $P = 0.015$) produced by zofenopril, suggesting that the benefit of zofenopril is, in part, a consequence of reduced oxidant stress in the endothelial cell leading to improved bioactive nitric oxide production.

4. Discussion

The present study demonstrates that administration of the sulfhydryl angiotensin-converting enzyme inhibitor zofenopril significantly improves nitric oxide production and bioactivity, and does so in conjunction with decreased endothelial cell oxidant stress in bovine aortic endothelial cells. These protective effects were significantly greater than those observed with the sulfhydryl angiotensin-converting enzyme inhibitor captopril and occurred at lower doses of the drug. The non-sulfhydryl angiotensin-converting enzyme inhibitor enalapril did not exhibit these beneficial effects. Thus, our data demonstrate that zofenopril is a unique angiotensin-converting enzyme inhibitor compared with captopril and enalapril. The biochemical and molecular bases for this unique mechanism is not entirely clear and will be the focus of another study.

Multiple pathogenic mechanisms in the development of atherosclerosis are offset by the nitric oxide pathway (Loscalzo, 2001; Napoli and Ignarro, 2001). Thus, the activation of the nitric oxide pathway by angiotensin-converting enzyme inhibitors may play an important role in its antiatherogenic effects (Linz et al., 1995). Our study supports an important role for NO-related protective effects of angiotensin-converting enzyme inhibition in endothelial cells. This protection could be related to the higher sulfhydryl-mediated free-radical scavenging activity of zofenopril compared to captopril (Cushman et al., 1989; Chopra et al., 1992), an action that may also reflect its greater lipophilicity than captopril. Sulfhydryl compounds as a class have antioxidant effects (Simic, 1988), being able to neutralize oxygen radicals by either a hydrogen donating or electron transferring mechanism (Simic, 1988; Mak et al., 1990). The mechanism of oxygen radical scavenging mediated by sulfhydryl compounds may also involve carbon-centered radical production. It also appears that the protective effects of sulfhydryl agents correlate better with their direct hydroxyl radical scavenging abilities than with their antiperoxidative potency (Simic, 1988; Mak et al., 1990). These considerations are further supported by the fact that in the present study the non-sulfhydryl angiotensin-converting enzyme inhibitor enalapril did not exibit any antioxidant effects. The antioxidant effects of zofenopril and captopril, but not enalapril, were also reported in vivo in apolipoprotein E-deficient mice (de Nigris et al., 2001). In addition, it has previously been reported that captopril is very effective in scavenging free radicals, in a manner similar to glutathione, *N*-2-mercaptoproppionylglycine, and *N*-acetylcysteine,

but this effect was not mimicked by enalapril (Goldschmidt and Tallarida, 1991). Further support for this view is provided by the fact that zofenopril exerted cardioprotective effects on doxorubicin-induced cardiotoxicity in the rat, which is also an oxygen radical-mediated process (Sacco et al., 2001). A reduction of the expression of oxidation-sensitive nuclear factor kappa B-dependent proinflammatory factors by angiotensin-converting enzyme inhibitors has also been reported in atherosclerotic rabbits (Hernandez-Presa et al., 1998). We have found that isoprostanes are decreased by sulfhydryl angiotensin-converting enzyme inhibitors. This observation is particularly relevant to the main purpose of this study as isoprostanes are produced from polyunsaturated fatty acids through radical-catalyzed mechanisms and represent a reliable in vivo marker of oxidative stress (Cracowski et al., 2002).

The beneficial action of angiotensin-converting enzyme inhibition is here broadened to the promotion of endothelial function and health. Indeed, the Prevention of Atherosclerosis with Ramipril-2 collaborative research group (MacMahon et al., 2000) suggested that beneficial effect of angiotensin-converting enzyme inhibitors on major coronary events may be due to reversal of endothelial dysfunction. Further studies are needed to address this issue.

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