Contribution of Captopril Thiol Group to the Prevention of Spontaneous Hypertension

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Summary

We aimed to compare the effect of angiotensin converting enzyme (ACE) inhibitors captopril (containing thiol group) and enalapril (without thiol group) on the development of spontaneous hypertension and to analyze mechanisms of their actions, particularly effects on oxidative stress and NO production. Six-week-old SHR were divided into three groups: control, group receiving captopril (50 mg/kg/day) or enalapril (50 mg/kg/day) for 6 weeks. At the end of experiment, systolic blood pressure (SBP) increased by 41% in controls. Both captopril and enalapril prevented blood pressure increase, however, SBP in the captopril group (121±5 mmHg) was significantly lower than that in the enalapril group (140±5 mmHg). Concentration of conjugated dienes in the aorta was significantly lower in the captopril group compared to the enalapril group. Captopril and enalapril increased NO synthase activity in the heart and aorta to the similar level. Neither captopril nor enalapril was, however, able to increase expression of eNOS. Both ACE inhibitors increased the level of cGMP. In the aorta, however, cGMP level was significantly higher in the captopril group. We conclude that captopril, beside inhibition of ACE, prevented hypertension by increasing NO synthase activity and by simultaneous decrease of oxidative stress which resulted in increase of cGMP concentration.

Key words: spontaneous hypertension, captopril, enalapril, nitric oxide synthase, thiols, reactive oxygen species
Introduction

Angiotensin converting enzyme (ACE) inhibitors are well established drugs in the treatment of hypertension (Williams 1988, Šimko and Šimko 1999, 2000) and protection of the cardiovascular system during congestive heart failure (Šimko 2002, Šimko et al. 2003). Although the pharmacological mechanisms of ACE inhibitors are not fully understood, various studies suggest that these agents may, except inhibition of angiotensin II production, stimulate synthesis of prostaglandin and bradykinin, inhibit production of superoxides, scavenge free radicals, and increase expression of endothelial nitric oxide synthase (eNOS), (Mak et al. 1990, Pecháňová et al. 1997, 2006, Kojšová et al. 2006). Moreover captopril and enalapril were shown to have direct effects on pathological voltage-operated calcium channel in cardiac myocytes (Wang et al. 1996).

Captopril, in contrast to enalapril, is the ACE inhibitor involving thiol group, which might participate in thiols formation and therefore it may have stronger antioxidant effect than other ACE inhibitors. Nevertheless, there are concerns with respect to the intensity of antioxidant effects of thiol-group-containing and non-thiol ACE inhibitors (Bartosz et al. 1997, Šimko et al. 2002a,b). Clearfield et al. (1994) documented that captopril and enalapril were approximately equally active in the prevention of LDL oxidation. Both these ACE inhibitors were shown to increase similarly the activity of superoxide dismutase and glutathione peroxidase in the liver (de Cavanagh et al. 1995) as well as Na+/K+-ATPase and Ca²⁺-ATPase activities in erythrocytes (Golik et al. 1996). However, Temel and Akyuz (2007) reported that captopril had an inhibitory effect on red cell plasma membrane Na+/K+-ATPase activity. Aznaouridis et al. (2007) showed that acute renin-angiotensin system
inhibition with quinapril may be more beneficial in terms of arterial function and central hemodynamics compared to captopril. On the other hand, only captopril, but not enalapril, improved the endothelium-dependent and independent relaxation responses in the isolated rat aorta of obese Zucker rats (Duarte et al. 1999). Analogically, while collagen-induced platelet aggregation was significantly reduced by captopril, no change was seen with enalapril (Skowasch et al. 2006). Moreover, captopril in contrast to non-thiol group ACE inhibitors was able to protect the heart against arrhythmias induced by ischemia/reperfusion (van Gilst et al. 1986).

The aim of this study was to compare the effect of captopril and enalapril on the development of hypertension in spontaneously hypertensive rats (SHR) and to analyze mechanisms of their actions, particularly effects on oxidative stress, NO synthase activity, eNOS protein expression, and cGMP level in the heart and aorta.

Methods

Chemicals and drugs
All the chemicals used were purchased from Sigma Chemicals Co. (Germany) except of $[^3]$H-L- arginine (Amersham, UK).

Animals and treatment
All procedures and experimental protocols were approved by the Ethical Committee of the Institute of Normal and Pathological Physiology SAS, and conform to the European Convention on Animal Protection and Guidelines on Research Animal Use. Young 6-week-old male spontaneously hypertensive rats (SHR) were randomly divided into water drinking control group and groups receiving captopril or enalapril
in the dose of 50 mg/kg/day in tap water for 6 weeks (n=7 in each group). All animals were housed in the room with a stable temperature of 23±1 °C and fed a regular pellet diet ad libitum. Daily water consumption was estimated individually for every animal one week before the experiment. During the experiment, drinking fluid consumption was controlled and adjusted, if necessary. Systolic blood pressure (SBP) was measured by non-invasive tail-cuff plethysmography every week. After 6 weeks of treatment, the animals were sacrificed and body weight (BW) and heart weight (HW) were determined. The HW/BW was calculated. Concentration of conjugated dienes (CD, the marker of membrane oxidative damage), total NO synthase activity, expression of eNOS protein, and cGMP level were determined in the heart and aorta.

**Conjugated dienes concentration**

The concentration of CD was measured in lipid extracts of the heart and aorta homogenates according to Kogure et al. (1982). Briefly, after chloroform evaporation under the inert atmosphere and addition of cyclohexane, CD concentration was determined spectrophotometrically (λ = 233 nm, Bio-Rad, GBC 911A).

**Total NO synthase activity**

Total NO synthase activity was determined in crude homogenates of the heart and aorta by measuring the formation of L-[³H]citrulline from L-[³H]arginine (Amersham, UK) as previously described by Bredt and Snyder (1990) with minor modifications (Pecháňová et al. 1997).
Western blot analysis of eNOS

Samples of the heart and aorta (40 mg of wet tissue) were homogenized in 25 mmol/l Tris-HCl, pH 7.4, containing 5 mmol/l EDTA, 50 mmol/l NaCl, 1 μmol/l leupeptin, 0.3 μmol/l aprotinin, 0.1 mmol/l PMSF, 1 mmol/l pepstatin and 1% SDS. After the centrifugation (15000 x g, 20 min, twice) supernatants were subjected to SDS-PAGE using 10% gels. Following the electrophoresis, proteins were transferred to nitrocellulose membranes and were probed with a polyclonal rabbit anti-endothelial NO synthase (Alexis Biochemicals, Germany). Bound antibody was detected using a secondary peroxidase-conjugated anti-rabbit antibody (Alexis Biochemicals, Germany). The bands were visualized using the enhanced chemiluminescence system (ECL, Amersham, UK) and analyzed densitometrically using Photo-Capt V.99 software.

Cyclic GMP concentration

Cyclic GMP concentration was measured in the heart and aorta using commercial available radioimmunoassay kit (Immunotech, S.A., France) based on the competition between succinylated cGMP of the sample and \(^{125}\text{I}\) labelled tracer for binding to polyclonal antibody coated onto tubes. Concentration of the cGMP in the sample was calculated from the standard curve and expressed as pmol/g tissue.

Statistical Analysis

The results are expressed as mean ± SEM. One-way analysis of variance and Bonferroni test were used for statistical analysis. Values were considered to differ significantly if the probability value was less than 0.05.
Results

Blood pressure and cardiac hypertrophy

In young SHR, the chronic captopril and enalapril treatment prevented BP increase occurring during the development of spontaneous hypertension. However, at the end of experiment, the systolic blood pressure in the captopril group (121±5 mmHg) was significantly lower that that in the enalapril group (140±5 mmHg), (Fig. 1). Treatment with both ACE inhibitors reduced development of cardiac hypertrophy in young SHR similarly (Tab. 1).

Conjugated diene concentration

Figure 2 indicates that both captopril and enalapril treatment decreased the concentrations of conjugated dienes in the heart and aorta. In the aorta, however, CD concentration was significantly lower in the captopril group (335±19 nmol/g tissue) than in the enalapril group (380±24 nmol/g tissue), (Fig. 2).

Total NO synthase activity and eNOS protein expression

Chronic captopril and enalapril treatment increased total NO synthase activity in the heart and aorta to the similar level (Fig. 3A). Neither captopril nor enalapril was, however, able to increase expression of eNOS protein in the tissues investigated (Fig. 3B).

Cyclic GMP concentration

Figure 4 shows that both captopril and enalapril treatment increased the levels of cGMP in the heart and aorta. In the aorta, however, the level cGMP was significantly
higher in the captopril group (2.94±0.21 nmol/g tissue) than in the enalapril group (2.30±0.15 nmol/g tissue), (Fig. 4).

Discussion

This is the first report documenting that chronic captopril treatment is more effective in the prevention of spontaneous hypertension than enalapril treatment. Both ACE inhibitors increased NO synthase activity in the heart and aorta to the similar level. Noteworthy, captopril was able to decrease oxidative stress and to increase cGMP level in the aorta more efficiently than enalapril. Thus, we hypothesise that captopril thiol group may contribute to the beneficial effect of captopril in the prevention of hypertension.

Both ACE inhibitors decreased oxidative damage measured as CD concentration in the heart and aorta. While similar effect of both drugs was detected in the heart, captopril was more effective than enalapril in the aorta. From figure 2 it is evident that in spontaneously hypertensive rats CD concentration was higher in the aorta than in the heart. Supposedly, the active NAD(P)H oxidase in the endothelial and smooth muscle cells of the aorta (Zalba et al. 2001, Touyz and Schiffrin 2004) contributes to this difference. ACE inhibitors, concomitantly with decreasing angiotensin II production, were shown to inhibit NAD(P)H oxidase activity (Touyz and Schiffrin 2004). Our experiment demonstrated that they are also potent activators of NO synthase. Nevertheless, neither captopril nor enalapril was able to increase expression of eNOS protein in the tissues investigated. Thus, other mechanisms like stabilisation of dimeric form of NO synthase by reducing ROS are suggested to participate in NO synthase activation. Moreover, the thiol group of captopril may
protect NO from oxidation by scavenging free oxygen radicals (Lahera et al. 1993) and forming nitrosothiols (Myers et al. 1990), both effects could prolong NO half-life and potentiate its effect. Alternatively this protective effect of captopril could be due to the augmentation of the activity of NO synthase and cGMP cyclase which are thiol-dependent (Ignarro et al. 1981). All the above activities are more expressed within vessels than heart, thus captopril may be more effective in the aorta.

In our experiment, treatment with both ACE inhibitors reduced development of cardiac hypertrophy in young SHR to the similar level. Probably, the blood pressure difference between SHR treated with captopril and enalapril (although significant during last three weeks) was too small for producing significant change in the reduction of cardiac hypertrophy. In agreement with our study, the ACE inhibitors captopril and enalapril reversed similarly the cardiovascular complications (cardiac hypertrophy, proteinuria) in the obese Zucker rat, an animal model of non-insulin-dependent diabetes mellitus (Duarte et al. 1999). On the other hand, only captopril, but not enalapril, improved the impaired endothelium-dependent and independent relaxation responses in the isolated rat aorta of the obese Zucker rat (Duarte et al. 1999). Captopril was also able to increase relaxation responses of femoral artery isolated from L-NAME hypertensive rats (Sládková et al. 2007). Miller et al. (2007) demonstrated that captopril, similarly like NAD(P)H oxidase inhibitor apocynin or superoxide dismutase mimetic tempol, potentiated luminal expansion in collaterals of spontaneously hypertensive rats. However, enalapril did not promote luminal expansion.

In our previous works we have also shown, that captopril treatment in the dose 100 mg/kg/day prevented the increase in blood pressure and left ventricular hypertrophy due to chronic NO synthase inhibition (Pecháňová et al. 1997) and
reversed the cardiovascular changes in already developed L-NAME-induced hypertension (Bernátová et al. 1999, 2000). Captopril-induced lowering of sympathetic tone was suggested to be mainly involved in these effects (Zicha et al. 2006a, Hojná et al. 2007). Chronic treatment of hereditary hypertriglyceridemic rats with captopril along with preserving normal blood pressure prevented also the impairment of vascular relaxation (Šimko et al. 2002a, Török et al. 2002, 2007; Zicha et al. 2006b).

Except the rat models, there is also evidence that ACE inhibitors with thiol group induce sustained reduction of systemic oxidative stress and improve the nitric oxide pathway in patients with essential hypertension (Napoli et al. 2004). It was demonstrated that after 12-week treatment, malondialdehyde levels were significantly reduced by zofenopril (ACE inhibitor containing thiol group) but not by enalapril. Similarly, isoprostanes were normalized after zofenopril treatment, whereas enalapril was ineffective (Napoli et al. 2004).

Recently we also demonstrated that chronic administration of N-acetylcysteine (NAC, antioxidant with thiol group) partially attenuated the blood pressure increase occurring in young spontaneously hypertensive rats. Mechanisms responsible for blood pressure reduction appear to be related to both the decrease of reactive oxygen species level and the increase of NO production (Pecháňová et al. 2006, 2007). While NAC attenuated the blood pressure increase only partially, captopril treatment was able to keep the blood pressure of young SHR on the level of normotensive rats.

In conclusion, it seems that the simultaneous effect of captopril on ACE inhibition, the decrease of oxidative stress and increase of NO synthase activity with
increased concentration of cGMP led to complete prevention of hypertension in young SHR.

Acknowledgement

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References


Table 1 Body weight (BW) and relative heart weight (HW/BW) in control SHR and SHR treated with captopril and enalapril for 6 weeks.

<table>
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<tr>
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<th>SHR controls</th>
<th>SHR + captopril</th>
<th>SHR + enalapril</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>BW (g)</td>
<td>311.7±10.2</td>
<td>287.4±12.9</td>
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<td>HW/BW (mg/g)</td>
<td>3.25±0.07</td>
<td>2.36±0.11*</td>
<td>2.48±0.10*</td>
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Data are means ± S.E.M., Significant differences: * p<0.05 compared to SHR controls.
Figure captions

**Figure 1.** Effect of 6-week captopril and enalapril treatment on systolic blood pressure of SHR. Control SHR (CONT), enalapril (ENAP), captopril (CAP), significant differences: * p<0.05 compared to SHR controls, + p<0.05 compared to enalapril treated SHR.

**Figure 2.** Effect of 6-week captopril and enalapril treatment on conjugated diene (CD) concentration in the heart and aorta. Control SHR (CONT), enalapril (ENAP), captopril (CAP), significant differences: * p<0.05 compared to SHR controls, + p<0.05 compared to enalapril treated SHR.

**Figure 3.** Effect of 6-week captopril and enalapril treatment on NO synthase activity and eNOS protein expression in the heart and aorta. Control SHR (CONT), enalapril (ENAP), captopril (CAP), significant differences: * p<0.05 compared to SHR controls, + p<0.05 compared to enalapril treated SHR.

**Figure 4.** Effect of 6-week captopril and enalapril treatment on cGMP concentration in the heart and aorta. Control SHR (CONT), enalapril (ENAP), captopril (CAP), significant differences: * p<0.05 compared to SHR controls, + p<0.05 compared to enalapril treated SHR.
Figure 1

The figure shows a graph plotting blood pressure (in mmHg) against weeks. Three groups are compared: CONT, ENAP, and CAP. The graph indicates a significant increase in blood pressure over time for all groups, with the CONT group having the highest blood pressure levels. The CAP group shows a slight decrease in blood pressure compared to the other two groups. The vertical bars represent the standard error of the mean, and the asterisks indicate statistically significant differences between groups and time points.
Figure 2

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![Bar graphs showing the concentration of CD (nmol/g tissue) in different tissues under different conditions.](image-url)
Figure 3

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