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MINIREVIEW

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## The Effect of Different Antioxidants on Nitric Oxide Production in Hypertensive Rats

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### Summary

The imbalance between nitric oxide (NO) and reactive oxygen species (ROS) production appears to be a common feature of experimental and human hypertension. Previously, different antioxidants and/or scavengers of oxygen free radicals were shown to activate nitric oxide synthase (NO synthase, NOS) and to increase the expression of both endothelial and neuronal NO synthase isoforms leading to blood pressure reduction. On the other hand, various antihypertensive drugs have been documented to possess antioxidant properties, which may contribute to their beneficial effect on blood pressure. This review is focused on the effects of antioxidant treatment in different models of experimental hypertension with a special attention to the prevention of oxidative damage and the augmentation of NO synthase activity and expression of NOS isoforms.

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### Key words

N-acetylcysteine • Melatonin • Provinols™ • Apocynin • Aspirin • Indapamide • Captopril • Spironolactone

### Introduction

In various models of hypertension, impaired endothelium-dependent relaxations have been described implying an endothelial dysfunction and an apparent decrease in the production of bioactive nitric oxide (Tschudi *et al.* 1996). The increased release of endothelial vasoconstricting factors such as thromboxane A<sub>2</sub>, endothelins and endoperoxides as well as production of superoxide anions may explain this endothelial

dysfunction in hypertension. Indeed, enhanced formation of endothelial superoxide anion (O<sub>2</sub><sup>-</sup>) has been described in vessels of rats with experimental hypertension (Rajagopalan *et al.* 1996, Noll *et al.* 1997, Pecháňová *et al.* 1999, Zicha *et al.* 2001, Kuneš *et al.* 2004). Such an increase in O<sub>2</sub><sup>-</sup> production accelerates the inactivation of NO and accounts for the apparent decrease in bioactive NO. Although increased NO synthase activity has been documented in various models of experimental hypertension, the level of cyclic guanosine

monophosphate (cyclic GMP), a marker of NO efficiency, is similar to normotensive control or even decreased in hypertensive animals. This discrepancy indicates that endogenously produced NO in spontaneous hypertension is not able to rise the cyclic GMP level adequately probably due to the physical barrier such as fibrotic intimal layer of hypertensive vessels and/or due to the increased oxidative stress (Noll *et al.* 1997, Pecháňová *et al.* 2006a). The imbalance between NO and  $O_2^-$  production appears to be a common feature of experimental and human hypertension.

The increased production of the reactive oxygen species may finally lead to target organ damage. Antioxidant treatment may thus prevent or reduce the hypertension and associated organ alterations. The review is focused on the effects of antioxidant treatment on the prevention of oxidative damage, on NO synthase activity and on the expression of NOS isoforms (particularly endothelial NOS) in different models of experimental hypertension.

### Antioxidant treatment in hypertension

In hypertensive models such as spontaneously hypertensive rats (SHR), DOCA-salt and angiotensin II infusion, blood pressure is reduced and vascular remodeling is inhibited by a diet rich in vitamin C or E (Chen *et al.* 2001). In these forms of hypertension, tempol (superoxide dismutase (SOD) mimetic) decreases blood pressure and improves endothelium-dependent relaxation, media/lumen ratio, NO synthase activity, kidney damage and glomerular filtration (Chen *et al.* 2001, Kawada *et al.* 2002). In young but not in adult Dahl salt-sensitive rats the tempol pretreatment also augmented blood pressure (BP) response to subsequent NO synthase inhibition, indicating increased NO bioavailability after lowering superfluous superoxide levels (Zicha *et al.* 2001). Likewise, overexpression of SOD and catalase reduces hypertension, increases availability of NO and endothelium-dependent relaxation in different models of hypertension (Chu *et al.* 2003). Apocynin (NAD(P)H oxidase inhibitor) also prevents blood pressure elevation and cardiovascular hypertrophy in aldosterone-infused rats (Park *et al.* 2004).

In NO-deficient hypertension, the acceleration of blood pressure recovery by treatment with antioxidant Provinols™ was associated with increased NO synthase activity (Bernátová *et al.* 2002). In addition, the simultaneous administration of Provinols™ with NO

synthase inhibitor N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) led to the prevention of NO synthase inhibition and partial attenuation of hypertension development (Pecháňová *et al.* 2004a). It seems that a treatment with ACE inhibitors or angiotensin II receptor antagonists may also lead to the correction of the NO/  $O_2^-$  imbalance since ramipril increased NO production and decreased superoxide accumulation in spontaneously hypertensive rats (Wiemer *et al.* 1997) and angiotensin II type 1 receptor antagonist CS-866 prevented an increase of redox-sensitive transcription factor nuclear factor  $\kappa$ B (NF- $\kappa$ B) in NO-deficient hypertension (Kitamoto *et al.* 2000). In this context, the thiol group of ACE inhibitor captopril may also contribute to the improvement of the redox state in different types of hypertension (Pecháňová *et al.* 1997, 2006b). Moreover, aldosterone receptor blocker, spironolactone, and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, simvastatin, are able to increase NO synthase activity with a simultaneous decrease of ROS concentration, which finally leads to the attenuation of hypertension (Pecháňová *et al.* 2006b, Šimko *et al.* 2004).

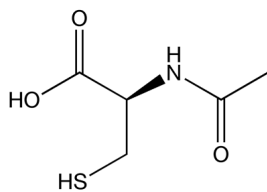
Since our group has longer experience with the antioxidant effects of N-acetylcysteine, melatonin, Provinols™, apocynin, aspirin, indapamide, and captopril in different forms of hypertension, we summarize here the antioxidant and scavenging properties of the above drugs and their effects on NO synthase activity and expression.

### Scavengers and antioxidants

#### *N-acetylcysteine*

N-acetylcysteine (NAC), derived from the simple amino acid cysteine (Fig. 1), provides significant protection against a broad array of toxins. N-acetylcysteine is a vital antioxidant, which beneficial characteristics include the scavenging of potent hydroxyl radicals and diminished production of hydrogen peroxide (Aruoma *et al.* 1989). In addition, N-acetylcysteine helps the body to convert and to synthesize glutathione, an amino acid compound that consists of glycine, L-glutamic acid, and L-cysteine, and is found in every cell. While glycine and L-glutamic acid are plentiful in our diets, the amount of glutathione, which our bodies can produce is limited by their store of cysteine. Supplementation with N-acetylcysteine thus helps the body to produce glutathione at more beneficial level against oxidative systems (Cabassi *et al.* 2001).

Pecháňová *et al.* (2006a) have documented that chronic effect of NAC administration on blood pressure in SHR is dependent on the stage of hypertension development. Chronic administration of N-acetylcysteine partially attenuated the blood pressure increase occurring in young spontaneously hypertensive rats. On the contrary, the effect of NAC was negligible in adult SHR with fully developed hypertension. The same was true for the age-dependent effect of chronic NAC administration on cardiac hypertrophy, the effect being significant only in young but not in adult SHR. 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 indicated by the elevation of NO synthase activity and eNOS protein expression. Recently, we have also demonstrated that chronic NAC treatment prevented the development of L-NAME-induced hypertension in adult WKY rats. This effect was associated with increased NOS activity and enhanced NO-dependent vasodilation (Rauchová *et al.* 2005, Zicha *et al.* 2006). NAC treatment also increased NO synthase activity in the developed form of L-NAME-induced hypertension, but without lowering blood pressure, i.e. similarly as in the developed form of spontaneous hypertension (Rauchová *et al.* 2005). Thus, increasing NO production, similarly as decreasing ROS generation, seems to be more beneficial in the prevention of hypertension when possible secondary changes (such as pronounced remodeling of resistance vessels) are still absent.

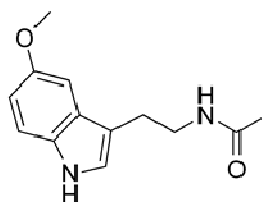


**Fig.1.** Structural formula of NAC

Nevertheless, chronic treatments with NAC improved the maximal relaxation of mesenteric arteries in adult SHR. Acute NAC treatment *in vitro* induced a relaxation of phenylephrine-precontracted arteries that was more pronounced in SHR than in WKY and was not abolished by L-NAME (Girouard *et al.* 2003). On the contrary, Pecháňová *et al.* (to be published) also documented NO-dependent vasodilator effect of NAC on femoral artery isolated from adult SHR. NAC was also shown to potentiate the antihypertensive response to angiotensin-converting enzyme (ACE) inhibitors in SHR

and hypertensive patients by a nitric oxide-dependent mechanism (Ruiz *et al.* 1994, Barrios *et al.* 2002).

Finally, Ramasamy *et al.* (1999) demonstrated that N-acetylcysteine increased endothelial NOS (eNOS) expression in cultured bovine aortic endothelial cells on both mRNA and protein levels and increased NO synthase activity. Thus, the mechanisms of increased NO production by NAC treatment include increased expression of eNOS mRNA and protein which leads to increased NO synthase activity. It is evident that NAC may increase NO synthase activity by stabilization of its dimeric form due to decreased ROS level. NAC may also protect already synthesized NO from oxidation by scavenging oxygen-free radicals (Lahera *et al.* 1993), and by forming nitrosothiols (Myers *et al.* 1990). Both effects could prolong NO half-life and potentiate its effect. The increased production of nitric oxide in developed form of hypertension is, however, less functionally effective due to either inactivation of nitric oxide by ROS, simultaneous release of endothelium-dependent vasoconstrictors or due to anatomical changes such as the hypertension-induced intimal thickening, which attenuates NO action on vascular smooth muscle cells.



**Fig. 2.** Structural formula of melatonin

#### Melatonin

Melatonin, 5-methoxy-N-acetyltryptamine (Fig. 2), is secreted by the pineal gland in the brain and is important in the regulation of many hormones in the body. It is naturally synthesized from the amino acid tryptophan by the enzyme 5-hydroxyindole-O-methyltransferase. Among its key roles, melatonin controls the body circadian rhythm, an internal 24-hour time-keeping system. Although the primary site of melatonin action is *via* the melatonin receptors, melatonin is a powerful antioxidant that can easily cross cell membranes and the blood-brain barrier (Hardeland 2005). Unlike other antioxidants, melatonin does not undergo redox cycling, the ability of a molecule to undergo reduction and oxidation repeatedly. Melatonin, once oxidized, cannot be reduced to its former state because it forms several stable end-products upon reacting with free radicals. Therefore, it has been referred to as a terminal

antioxidant (Tan *et al.* 2000).

Melatonin treatment, similarly as NAC treatment, partially attenuated BP rise in young SHR, which was accompanied by increased NO synthase activity in the heart and kidney. In contrast to NAC, melatonin was not able to enhance endothelial NO synthase protein expression. Neither NAC nor melatonin had any effect on the protein expression of inducible NO synthase (iNOS). Melatonin treatment further lowered ROS concentrations as evidenced by decreased conjugated diene concentration and NF- $\kappa$ B expression. It seems that both increased NO synthase activity and ROS reduction are responsible for preventive effects of melatonin on the development of spontaneous hypertension. While NAC was able to enhance NO synthase activity also *via* the increased expression of endothelial NO synthase protein, melatonin has probably direct effect(s) on NO synthase activity (Pecháňová *et al.* 2004b).

Whereas NAC had no significant effect on BP of adult SHR, chronic melatonin treatment decreased BP significantly. Analogically to the preventive experiment, melatonin increased NO synthase activity without up-regulation of endothelial or inducible NO synthase protein expression. It lowered conjugated diene concentration and attenuated NF- $\kappa$ B expression, indicating a decrease in ROS production. Both agents NAC and melatonin were able to increase NO synthase activity and reduce ROS, however, only melatonin decreased BP of adult SHR (Pecháňová *et al.* 2006c, Paulis *et al.* 2005). Additional mechanisms, different from increased NO synthesis or decreased ROS production, may be involved in this blood pressure lowering effect of melatonin. Melatonin binding to its receptors leading to changes in the regulation of calcium and potassium channels and/or direct activation of guanylate cyclase can be supposed (Pogan *et al.* 2002). Melatonin receptors have been found on vascular smooth muscle cells (Capsoni *et al.* 1994) as well as on endothelial cells (Masana *et al.* 2002). In agreement with *in vivo* studies, our *in vitro* experiment indicated that the melatonin-induced relaxation was only partially inhibited by NOS inhibitor L-NAME administration with an additive effect of soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), suggesting the presence of a significant NO-dependent component. Nevertheless, melatonin-induced relaxation was still preserved after a combined L-NAME plus ODQ administration. This *in vitro* finding indicates that

additional mechanisms different from the improvement of NO pathway (e.g. melatonin receptor-mediated vasodilation) may be involved in the blood pressure lowering and vasorelaxant effects of melatonin (Pecháňová *et al.* 2006c).

#### *Provinols™*

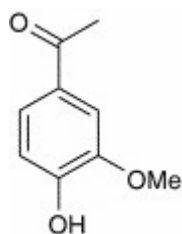
Many epidemiological studies have shown that regular flavonoid intake in grape juice, red wine and in some other beverages is associated with reduced risk of cardiovascular diseases (Fuster *et al.* 1992, Middleton *et al.* 2000, Zenebe *et al.* 2003, Curin and Andriantsitohaina 2005). Indeed, these compounds including the red wine polyphenolic compounds (Provinols™) possess a number of biological effects that might participate in vascular protection, including anti-aggregatory platelet activity, and antioxidant and free radical scavenging properties. Another therapeutically relevant effect of polyphenols may be their ability to interact with the generation of NO from vascular endothelium, which leads not only to vasodilatation, but also to the expression of genes which protect the cardiovascular system (Diebolt *et al.* 2001, Zenebe and Pecháňová 2002, Bernátová *et al.* 2002, Pecháňová *et al.* 2004a, Duarte *et al.* 2004). Also, polyphenols contribute to the preservation of the integrity of cells belonging to the vascular wall, mainly those in the endothelium, by acting on the signaling cascades implicated in endothelial apoptosis. Due to their antioxidant properties, diets supplemented with foods containing polyphenols, might also protect different tissues against ischemic damage. Polyphenols reduce oxidative and nitrosative stresses leading to cellular death. All these effects of polyphenols might interfere with atherosclerotic plaque development and stability, vascular thrombosis and occlusion and therefore might explain their vascular protective properties (Fitzpatrick *et al.* 1993, Andriambelison *et al.* 1997, 1999, Sulová *et al.* 2005, Jendeková *et al.* 2006a).

For more information see another minireview in this supplement (Pecháňová *et al.* 2006e).

#### *Apocynin*

A major source of reactive oxygen species in vascular cells is the NAD(P)H oxidase, membrane-associated enzyme that catalyse the one-electron reduction of oxygen using NADH or NADPH as the electron donor (Griendling *et al.* 2000). NAD(P)H oxidase consists of the membrane subunits gp91phox and p22phox and the cytosolic subunits p67phox, p47phox,

and the small GTPase rac1. The subunits assemble on activation and form the functional enzyme, which produces superoxide radicals after electron transfer to molecular oxygen (Wassmann *et al.* 2004). Apocynin (4-hydroxy-3-methoxyacetophenone) is a methoxy substituted catechol (Fig. 3), which has been used by Peruvian Indians as an anti-inflammatory agent. This compound was originally derived from the rhizome of the medicinal herb *Picrorhiza kurroa* found in the hilly sides of Himalayan highlands (Engels *et al.* 1992). It has been demonstrated to prevent translocation of p47phox and p67phox subunits from cytoplasm to membrane, and is therefore thought to prevent the assembly of NAD(P)H oxidase (Stolk *et al.* 1994). In this way apocynin effectively eliminates the increase of  $O_2^-$  production.

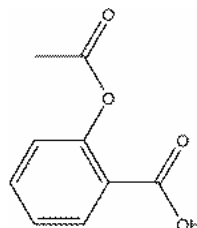


**Fig. 3.** Structural formula of apocynin

Hamilton *et al.* (2002) documented that the apocynin decreases  $O_2^-$  production in rat and human vascular rings, increases NO production in cultured human endothelial cells and improves endothelial function *ex vivo* in human arteries and veins as well as in arteries from stroke-prone spontaneously hypertensive rats. Furthermore, in aorta from spontaneously hypertensive rats treated with apocynin, i.e. under the conditions in which NAD(P)H oxidase-derived  $O_2^-$  production was inhibited and spontaneous tone was decreased, exogenous addition of  $O_2^-$  was able to restore the tone (Lodi *et al.* 2006). The treatment of basilar artery rings from OLETF rats (Otsuka Long-Evans Tokushima Fatty rats) with apocynin improved endothelium-dependent relaxation, L-NNA-induced contraction and ACh-induced cGMP production (Matsumoto *et al.* 2006). These results indicate that enhanced NAD(P)H oxidase activity and, hence, NAD(P)H driven superoxide production, is involved in the increase of spontaneous tone in experimental hypertension. Thus, the prevention of the decrease of NO level, although without increasing NO synthase activity, may partially attenuate the increase of blood pressure. In agreement with this hypothesis, we have demonstrated that apocynin significantly decreased

blood pressure rise in borderline hypertensive rats and lowered conjugated dienes (CD) concentration in the kidney and the expression of NF- $\kappa$ B in the left ventricle. Without affecting NOS activity in the left ventricle apocynin decreased the concentration of reactive oxygen species leading to the partial prevention of blood pressure rise in borderline hypertensive rats (Jendeková *et al.* 2006a). Similarly, in young SHR apocynin was able to decrease expression of NF-kappa B and to prevent cGMP decrease in the left ventricle, which led to a partial prevention of blood pressure (BP) rise (Jendeková *et al.* 2006b). On the other hand, apocynin was not able to decrease BP rise in the experimental model of L-NAME-induced hypertension (Pecháňová *et al.* 2006d).

Angermayr *et al.* (2006) demonstrated that chronic inhibition of NAD(P)H oxidase with apocynin significantly attenuated the formation of portosystemic collateral vessels and the development of hyperdynamic splanchnic circulation in portal hypertensive rats. These findings suggest that inhibition of NAD(P)H oxidase activity might also have therapeutic potential in the treatment of portal hypertension.



**Fig. 4.** Structural formula of aspirin

#### Aspirin

Aspirin (acetylsalicylic acid, ASA) (Fig. 4) is an antiplatelet agent, which inhibits platelet thromboxane  $A_2$  production, and therefore, thrombus formation. Aspirin has been shown to be effective for the primary and secondary prevention of atherothrombotic disease (Lip 2003). Its mode of action is the inhibition of cyclooxygenases, enzymes that catalyse the conversion of arachidonic acid to eicosanoids and thus may play an important role in platelet-vessel wall interactions (Rao 1993).

There is an extensive literature on the effects of aspirin in the prevention of cardiovascular events. Husain *et al.* (1998) have reported that improvement of endothelial dysfunction with aspirin may increase vasodilation, reduce thrombosis, and inhibit the progression of atherosclerosis. ASA is a potent

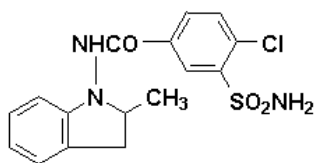
antioxidative agent that markedly reduces vascular production of superoxide in normotensive and hypertensive rats (Wu *et al.* 2002). Aspirin at the lower dose (30 mg/kg) protects the endothelium against damage elicited by low-density lipoprotein *in vivo*, and this protective effect is related to the reduction of nitric oxide synthase inhibitor level by increasing dimethylamino-hydrolase activity (Deng *et al.* 2004). Katsuyama *et al.* (1999) showed that aspirin dose-dependently inhibited cytokine-stimulated NO production and inducible NO synthase protein expression. In addition, ASA was found to prevent angiotensin-II-induced hypertension and cardiovascular hypertrophy, mainly through its antioxidative properties in preventing the generation of superoxide (Wu *et al.* 2004).

On the other hand, aspirin similarly like apocynin, failed to affect blood pressure rise in experimental L-NAME-induced hypertension (Pecháňová *et al.* 2006d). Despite that aspirin decreased ROS level measured as CD concentration in the kidney and total antioxidant capacity (TEAC) assay in the plasma, it did not affect NO synthase activity and fibrosis induced by L-NAME treatment. It is hypothesized that scavengers with activating effect on NO synthase and/or stabilizing effect on NO level are able to interfere successfully with L-NAME-induced hypertension. Decreasing ROS generation without simultaneous improvement of NO synthase activity has no beneficial effect on this form of hypertension (Pecháňová *et al.* 2006d).

## Antihypertensives with antioxidant effect

### Indapamide

Indapamide is an indole derivate of chlorobenzensulfonamide (Fig. 5) with diuretic properties (Chaffman *et al.* 1984). It differs chemically from the thiazides because it contains only one sulfonamide group and no thiazide ring. This gives the indapamide some specific properties. Indapamide has a direct vasodilator effect (Dollery 1991) and it also stimulates a synthesis of vasodilator PGE<sub>2</sub> and PGI<sub>2</sub> (Delbarre and Delbarre 1990).



**Fig. 5.** Structural formula of indapamide

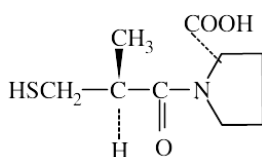
The antioxidant effect of indapamide has been suggested as well. Uehara *et al.* (1990) showed that indapamide enhanced the elimination of the stable free radical  $\alpha,\alpha$ -diphenyl- $\beta$ -picrylhydrazyl and reduced the formation of malondialdehyde in the rat brain homogenate. These authors demonstrated that indapamide lowered the oxidation of linolenic acid in the xanthine-xanthine oxidase system. Carey *et al.* (1996) demonstrated that six months of treatment with indapamide (2.5 mg daily) produced significant reductions in blood pressure and this effect was accompanied by regression of left ventricular hypertrophy. Reduction of relative left ventricular weight seems to be primarily due to reduction in ventricular wall thickness rather than chamber size. Experimental studies suggested that treatment with indapamide significantly reduced blood pressure and prevented the development of left ventricular or renal hypertrophy in hypertensive Dahl salt-sensitive rats (Uehara *et al.* 1993, Hayakawa and Raji 1997). Fixed low-dose combination treatment with perindopril/ indapamide for 8 weeks normalized the vasodilator response to acetylcholine in hypertensive Dahl salt-sensitive rats, thus improving endothelium-dependent (i.e. NO-dependent) relaxation. This finding is supported by the observation that nitric oxide synthase activity, which was lower in the aorta of hypertensive Dahl salt-sensitive rats, was restored by the perindopril/indapamide combination. Even the monotherapy with indapamide resulted in an increase of NOS activity (Hayakawa *et al.* 1997). This study is compatible with our findings, which revealed that treatment with indapamide increased NOS activity in the aorta and improved the average acetylcholine-induced vasodilatation of the femoral artery from spontaneously hypertensive rats (Kojšová *et al.* 2006a, Sládková *et al.* 2005). Indapamide treatment along with administration of ACE inhibitor captopril had the additive effect on the prevention of blood pressure increase in young SHR. On the other hand, this combination increased NOS activity in the aorta similarly as indapamide alone. Our results suggested that indapamide is responsible for NOS activity increase after the combined treatment with indapamide and captopril. This effect of indapamide may contribute to its vasorelaxant and antihypertensive properties (Kojšová *et al.* 2005b, 2006a, Jendeková *et al.* 2005). Furthermore, we have demonstrated that indapamide and hydrochlorothiazide (HCT) partially prevented blood pressure increase induced by L-NAME treatment. Moreover, indapamide increased the

expression of eNOS and nNOS as well as NO synthase activity inhibited by L-NAME treatment in the brain. We have documented that indapamide, even when used in ten times lower dose than HCT, prevented L-NAME-induced hypertension comparably. The contribution of indapamide-induced increase of brain NOS activity to this prevention is suggested (Kojšová *et al.* 2006b).

Benetos *et al.* (1995) reported that in the Dahl salt-sensitive rats long-term treatment with indapamide was able to improve the elastic properties of the carotid artery wall even in animals without salt loading. This finding supports the hypothesis that indapamide acted on the arterial wall independently of its natriuretic effect.

### Captopril

Captopril [1-[2(S)-3-mercapto-2-methyl-1-oxopropyl]-L-proline], an angiotensin-converting enzyme inhibitor (Fig. 6), is a commonly used antihypertensive drug, which also possesses antioxidant properties (Bartosz *et al.* 1997).



**Fig. 6.** Structural formula of captopril

Most of the clinical studies revealed that captopril not only decreases blood pressure but it also has vasodilator (Johns *et al.* 1984) and renoprotective effects (Manley 2000), and it attenuates left ventricular hypertrophy (Konstam *et al.* 2000, Creager and Roddy 1994, Conlin *et al.* 2000). Captopril was also shown to prevent or reverse left ventricular hypertrophy in different models of experimental hypertension (Pecháňová *et al.* 1997, Bernátová *et al.* 2000).

Captopril in the dose 100 mg/kg/day, but also 10 mg/kg/day, was able to prevent the development of hypertension as well as the increase of nucleic acid concentration and protein synthesis in the heart, aorta, brain and kidney when applied simultaneously with L-NAME (Pecháňová *et al.* 1997, Kojšová *et al.* 2005a). Captopril treatment caused total reversion of hypertension and LV hypertrophy and decreased the content of metabolic proteins, contractile proteins, and collagenous proteins compared to the L-NAME group

(Bernátová *et al.* 2000, Šimko *et al.* 2000). This protective effect of captopril was, however, not associated with increased NO synthase activity. Accordingly, captopril did not influence the reduction of cGMP concentration in this model of hypertension. While captopril did not affect the concentration of cGMP, it had more than the additive effect on the cAMP concentration increase in the cardiovascular system during long-term NO synthase inhibition (Pecháňová and Bernátová 2000). Recently, Zicha *et al.* (2006) demonstrated that chronic captopril administration to L-NAME-treated rats reduced sympathetic tone which is enhanced in this form of experimental hypertension (Pecháňová *et al.* 2004c).

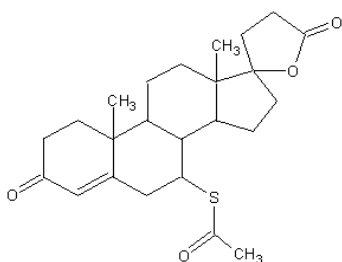
Treatment with captopril in the dose 10 mg/kg/day had no effect on NO synthase activity in the tissues of young SHR (Kojšová *et al.* 2006a). On the other hand, treatment with higher dose of captopril (100 mg/kg/day) significantly increased NO synthase activity in the heart of spontaneously hypertensive rats (Pecháňová and Bernátová 2001). Our recent experiments (Hojná *et al.* 2006, Zicha *et al.* 2006) revealed that a major part of antihypertensive effects of chronic captopril treatment in SHR are due to the reduction of sympathetic tone.

The protective effects of captopril against hypertension and oxidative damage may in part be related to the increase of specific activities of antioxidant enzymes such as the superoxide dismutases, glutathione peroxidase, and catalase (Cabell *et al.* 1997). Captopril is a scavenger of free radicals because of the presence of a sulfhydryl group. The scavenging action of captopril was examined against superoxide anion, hydroxyl radical, or hypohalite radical. Bagchi *et al.* (1989) reported that captopril is an extremely potent free radical scavenger, scavenging power being as effective as superoxide dismutase against superoxide anion, or dimethylthiourea against hydroxyl radical, but better than allopurinol against hypohalite radical. Andreoli (1993) demonstrated that captopril is able to scavenge hydrogen peroxide and prevent oxidant-induced cell injury. Finally, Zieden *et al.* (1995) showed that captopril increases the resistance of low-density lipoprotein to copper-induced oxidation. The treatment with captopril lowered the concentration of reactive oxygen species measured as decreased concentration of conjugated dienes in the kidney of spontaneously hypertensive rats (Pecháňová and Bernátová 2001, Pecháňová *et al.* 2005) and L-NAME-treated rats (Pecháňová and Capíková 2003). Previously we have also demonstrated that captopril increased the concentration of thiols in the renal tissue which may

contribute to the beneficial properties of this ACE inhibitor (Pecháňová *et al.* 2006b).

### Spirolactone

Spirolactone is a synthetic 17-lactone steroid (Fig. 7), which is a renal competitive aldosterone antagonist in a class of pharmaceuticals called potassium-sparing diuretics. It is used primarily to treat low-renin hypertension, hypokalemia, and Conn's syndrome. On its own, spironolactone is only a weak diuretic, but it can be combined with other diuretics. Spirolactone inhibits the effect of aldosterone by competing for intracellular aldosterone receptor in the distal tubule cells. This increases the secretion of water and sodium, while decreasing the excretion of potassium. Spirolactone has a fairly slow onset of action, taking several days to develop and similarly the effect diminishes slowly. Spirolactone is used to treat high blood pressure, congestive heart failure, kidney and liver disease and conditions in which there are abnormally low levels of potassium in the blood (Pitt *et al.* 1999).



**Fig. 7.** Structural formula of spironolactone

Although ACE inhibitors and angiotensin type 1 receptor blockers are well established drugs in the treatment and/or prevention of hypertension, they are supposed not to be sufficient in the inhibition of aldosterone formation. Therefore we analyzed the effect of aldosterone receptor blocker, spironolactone, on NO metabolism in the kidney of L-NAME treated rats. Besides the increase in systolic blood pressure and the decrease of NOS activity, L-NAME treatment resulted in the attenuated production of thiol and nitrosothiol group concentration in the different tissue. Simultaneous spironolactone treatment increased thiol group level and kept NOS activity and nitrosothiol group concentration on the control value (Pecháňová *et al.* 2006b). Thiol groups may protect NO molecule from oxidation by scavenging free radicals and forming nitrosothiols (Myers *et al.* 1990, Stamler *et al.* 1992). Both these effects can

prolong NO half-life and potentiate its vasorelaxant effect. In agreement, spironolactone improves endothelial dysfunction, increases NO bioactivity, and inhibits vascular angiotensin II production in patients with heart failure (Farquharson and Struthers 2000). In L-NAME-induced hypertension Pereira and Mandarin-de-Lacerda (2000) documented the preventive effect of spironolactone against increased blood pressure and diminution of vessel density in the heart. Moreover, spironolactone added to the ACE inhibitors normalized NO-mediated relaxation in experimental chronic heart failure by beneficial modulation of the balance between NO and superoxide anion formation and reduced blood pressure during development of diabetic hypertension (Liu *et al.* 2000, Farquharson and Struthers 2000). Furthermore, elevated systolic blood pressure in aldosterone-treated rats was partially suppressed by spironolactone or antioxidants such NAC and pyrrolidine dithiocarbamate (PDTC). Spirolactone, PDTC, and NAC each attenuated activation of NADPH oxidase and NF- $\kappa$ B expressed by endothelial cells and inflammatory cells in aldosterone-treated rats (Sun *et al.* 2002).

Spirolactone, in contrast to captopril, induced an increase in endothelial NOS protein expression. Although both spironolactone and captopril prevented the development of L-NAME-induced hypertension and reduction of thiol groups in the renal tissue, only spironolactone induced the increased expression of endothelial NOS protein. Increased expression of this NO synthase isoform may lead to the prevention of decreased NOS activity and nitrosothiol group concentration in the kidney (Pecháňová *et al.* 2006b). Both nitric oxide itself and S-nitrosothiols may contribute to the preventive effect of spironolactone against the development of hypertension.

### Conclusions

In this review we have documented that chronic effect of different antioxidants on blood pressure in experimental hypertension is dependent on the stage of hypertension development. While chronic administration of antioxidants partially attenuated the blood pressure increase occurring in young spontaneously hypertensive rats, the same antioxidants were less effective in adult SHR with fully developed hypertension. This was demonstrated using the antioxidants, which augmented NO synthase activity. Thus the increased production of nitric oxide in developed form of hypertension is less



functionally effective due to either inactivation of nitric oxide by ROS, simultaneous release of endothelium-dependent vasoconstrictors or due to anatomical changes such as the hypertension-induced intimal thickening, which attenuates NO action on vascular smooth muscle cells. Concerning L-NAME-induced hypertension, it is hypothesized that scavengers with activating effect on NO synthase and/or stabilizing effect on NO level are able to interfere successfully with this form of hypertension. Decreasing ROS generation without simultaneous improvement of NO synthase activity has no beneficial effect on L-NAME-induced hypertension.

Finally, antioxidant activity of different antihypertensives may also significantly contribute to their beneficial effects, i.e. blood pressure reduction and prevention of target organ damage.

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