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MINIREVIEW

Beneficial Effects of Provinols™: Cardiovascular System and Kidney

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Summary

Red wine polyphenols have been reported to exert beneficial effects in preventing cardiovascular diseases but their molecular mechanisms of hemodynamic effects on functional cardiovascular and renal changes were studied much less. The review is focused on *in vitro* as well as in *vivo* effects of red wine extract containing polyphenolic compounds (Provinols™) on cardiovascular systems and kidney in relation to the molecular and biochemical mechanisms of these compounds. This review provides the evidence that Provinols™ is able to produce *ex vivo* endothelium-dependent relaxation as a result of enhanced NO synthesis. Administration of Provinols™ partially prevents the development of hypertension during NO deficiency and accelerates the decrease of blood pressure in already established hypertension. The effects of Provinols™ include prevention and/or attenuation of myocardial fibrosis, reduction of aortic wall thickening and improvement of vascular functions. These functional and structural alterations are associated with significant augmentation of NO production, seen as the increase of NO synthase activity and eNOS protein expression. Moreover, it has been documented that Provinols™ decreased the oxidative stress within the cardiovascular system and kidney.

Key words

Red wine polyphenolic compounds • Nitric oxide • Vasorelaxation • Hypertension • Nephrotoxicity

Introduction

Many epidemiological studies have shown that regular intake of natural polyphenols 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). In general, more than two thirds of the polyphenols consumed in the diet are

flavonoids. Red wine flavonoids have generated a great interest especially due to their *in vivo* and *in vitro* antioxidant capabilities. Their beneficial effect on cardiovascular system was described mainly in relation to the French Paradox phenomenon as well as to the Mediterranean diet. The French Paradox is defined as a low incidence of coronary heart disease while consuming a diet rich in saturated fat. The Mediterranean diet, rich in fruits and red wine, was shown to protect against the development of cardiovascular diseases (Hertog *et al.* 1995, De Lorgeril *et al.* 1996). Consumption of fruits, vegetables and red wine may help to reverse hyperlipidemia, to decrease the atherogenicity of the LDL particles (Lampe 1999), and to protect LDL cholesterol from oxidation (Brouillard *et al.* 1997).

Grapes contain a wide variety of polyphenols including resveratrol (stilbene), catechins, flavonoids and its derivatives, flavons, flavonols, and anthocyanins. Indeed, these compounds present in the red wine possess a number of biological effects that might participate in vascular protection, including anti-aggregatory, antioxidant and free radical scavenging properties. Another therapeutically relevant effect of flavonoids 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 that protect the cardiovascular system (Middleton *et al*. 2000, Zenebe and Pecháňová 2002, Curin and Andriantsitohaina 2005). Polyphenols also 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 flavonoids, might also protect different tissues against ischemic damage. Flavonoids reduce oxidative and nitrosative stress leading to cellular death. All these effects of flavonoids might interfere with atherosclerotic plaque development and stability, vascular thrombosis and occlusion and they might therefore explain their vascular protective properties (Zenebe and Pecháňová 2002, Curin and Andriantsitohaina 2005, Andriantsitohaina *et al*. 2005). Recently, the possible advantage of a moderate wine consumption in patients with chronic renal failure was hypothesized (Caimi *et al.* 2004). Therefore, it is expected that the naturally occurring nutritional sources of antioxidants, such as fruits, vegetables, tea or wine, would also attenuate the renal damage caused by oxidative challenges.

This review is focused on the beneficial effects of red wine polyphenolic compounds (Provinols™) on the cardiovascular system and kidney.

1. Effect of Provinols™ on blood vessel relaxations

Polyphenolic compounds have been documented to relax precontracted smooth muscle of the arteries with intact endothelium. Moreover, some of them were also shown to relax endothelium-denuded arteries (Fuster *et al*. 1992, Andriambeloson *et al*. 1997). Several authors have reported that extracts from grape and wine induce endothelium-dependent relaxation *via* enhanced generation and/or increased biological activity of NO which leads to the elevation of cGMP level (Fitzpatrick *et al*. 1993, Andriambeloson *et al*. 1997). The increase in the intracellular Ca^{2+} level proceeds *via* a redox-sensitive pathway the activation of NO synthase, the production of NO and thus endothelium-dependent vasodilatation in different types of arteries from different species (Andriambeloson *et al*. 1999, Zenebe *et al*. 2003, Duarte *et al.* 2004).

Provinols™ represents the polyphenolic compounds isolated from red wine and it involves (in mg/g of dry powder) 480 proanthocyanidins, 61 total anthocyanins, 19 free anthocyanins, 38 catechin, 18 hydroxycinnamic acids, 14 flavonols and 370 polymeric tannins. It was documented that Provinols™ elicited endothelium-dependent relaxation of rat femoral artery by the Ca^{2+}-induced increase of NO synthase activity and by protecting NO from degradation (Zenebe *et al*. 2003). Because the action of red wine polyphenolic compounds has been associated with the improvement of endothelium-dependent relaxation and elevation of NO synthase activity and/or expression in several *in vitro* and *in vivo* experiments (Andriambeloson *et al.* 1998, Pecháňová *et al.* 2004a), it may be assumed about possible therapeutic effect of Provinols™ in diseases associated with reduced NO bioavailability such as endothelial dysfunction or atherosclerosis.

Zenebe *et al.* (2003) provided the evidence that Provinols™ elicited endothelium-dependent relaxation in rat femoral artery. The fact that the relaxation abolished by L-NAME was restored by L-arginine confirmed the involvement of NO in the endothelium-dependent vasorelaxation induced by Provinols™. Determination of NO synthase activity in the vascular tissue demonstrated that administration of Provinols™ at the concentration of

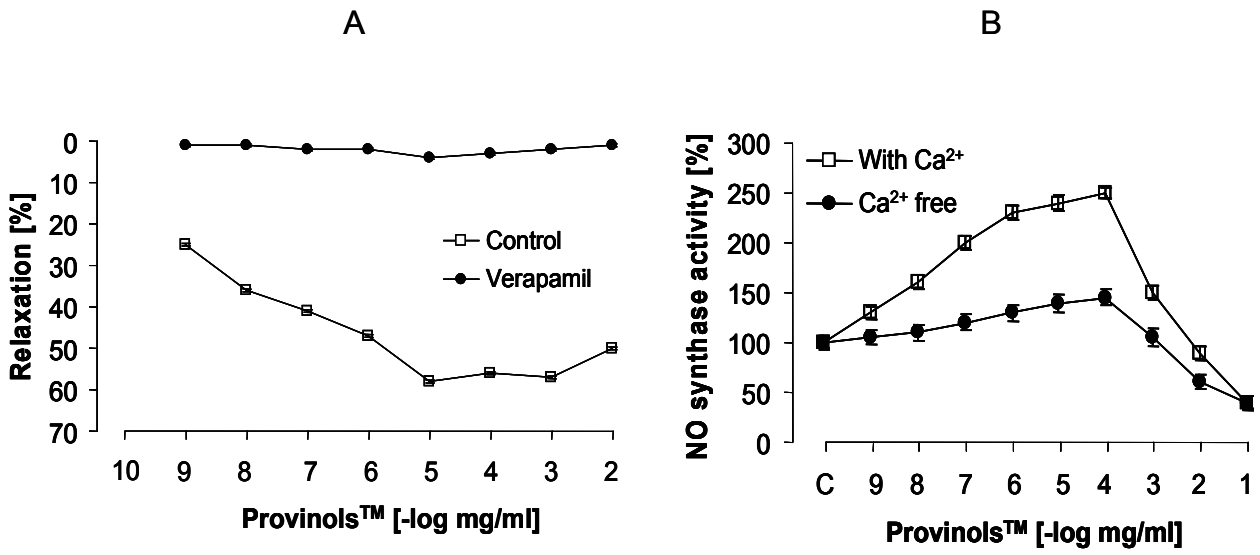

Fig. 1. Effect of the Ca^{2+}-entry blocker verapamil on the relaxation responses of femoral artery to Provinols™ administration. Responses before (□) and after (●) verapamil administration (A). NO synthase activity in the femoral artery after administration of cumulative doses of Provinols™. NO synthase activity in the presence (□) and without (●) Ca^{2+} (B). (adapted from Zenebe *et al.* 2003)

10^{-4} to 10^{-9} mg/ml increased the activity dose-dependently. The maximal activation of NO synthase was reached at the concentration of 10^{-4} mg/ml which correlated well with the maximal relaxation of the femoral artery induced by Provinols™ at the concentration of 10^{-5} mg/ml (Fig. 1 A,B).

Moreover, Provinols™ at the concentration producing the maximal endothelium-dependent relaxation, restored the relaxation of the femoral artery to acetylcholine abolished by superoxides and enhanced partially the relaxant responses of sodium nitroprusside suggesting the ability of Provinols™ to preserve NO from degradation (Zenebe *et al.* 2003, Pecháňová *et al.* 2006a). Similarly, red wine polyphenolic compounds caused a dose-dependent relaxation in rabbit aorta with intact endothelium (Cishek *et al.* 1997). The authors documented that relaxation responses were abolished by L-NAME and were associated with an increase in cGMP content. Flesch *et al.* (1998) also documented increased concentration in cGMP content in rat aortic rings after exposure to phenolic grape ingredients and red barrique wines. Since guanylate cyclase operates as an intracellular receptor for NO (Lancaster 1992), it is possible that increased concentration of NO was responsible for the enhancement of cGMP level, which was in agreement with the finding of increased NO synthase activity after Provinols™ administration. Adriambeloson *et al.* (1997) also demonstrated that some polyphenols induce endothelium-dependent relaxations *via* an enhancement of endothelial NO synthesis. The increase in intracellular concentration of Ca^{2+} ($[Ca^{2+}]i$) represents the critical step for the activation of NO synthase in the endothelial cells leading to the production of NO and the subsequent endothelium-dependent vasorelaxation (Lückhoff *et al.* 1988). This increase in $[Ca^{2+}]i$ can be due to either an influx of extracellular Ca^{2+} or a release of Ca^{2+} from intracellular stores. The relaxation produced by Provinols™ was completely prevented in the presence of the Ca^{2+}-entry blocker verapamil, suggesting that the Ca^{2+} influx to endothelial cells might be crucial for the relaxation ability of the red wine polyphenolic compounds (Zenebe *et al.* 2003). Analogically, Andriambeloson *et al.* (1999) reported that red wine polyphenolic compounds produced NO-dependent vasorelaxation of rat aortic rings through an extracellular Ca^{2+}-dependent mechanism. However, it cannot be excluded that a release of Ca^{2+} from intracellular stores might play a role in the endothelial NO-dependent relaxation produced by polyphenolic compounds. Indeed, after red wine polyphenolic compounds administration to the endothelial cell culture, Martin *et al.* (2002) documented an increase of $[Ca^{2+}]i$ from the intracellular stores, that was sensitive to the phospholipase C inhibitor.

Since Provinols™ is a mixture of different polyphenolic compounds, it is not certain which type of the phenolic components is responsible for the vasorelaxant responses. The molecular identity of the polyphenolic compounds responsible for this effect of Provinols™ probably belongs to oligomeric condensed

tannins and anthocyanins that were shown to mediate the *ex vivo* endothelial NO-induced relaxation of aortic rings (Andriambeloson *et al.* 1999). The combination of different phenolic compounds might also be the reason for the vasorelaxant effect of Provinols™, similarly to the reported antioxidant activity of a mixture of two flavonoids citrin and hesperdin (Scarbourough and Bacharach 1949, Kühnau 1976). Fitzpatrick *et al.* (1993) showed that monomers malvidine and resveratrol at concentrations up to 0.1 mM did not relax aortic rings while tannic acid produced endothelium-dependent relaxations that were inhibited by L-nitro-arginine. Quercetin produced only slowly developing relaxation in intact aortic rings, but relaxations were not affected by NO synthase inhibitor. Analogically, Duarte *et al.* (1993a,b) reported that the monomers catechin, epicatechin and quercetin did not show endothelium-dependent relaxation. Although Huang *et al.* (1999) demonstrated epicatechin-induced endothelium-dependent vasorelaxation in rat mesenteric arteries, it seems that polymeric rather than monomeric phenols were responsible for NO-dependent relaxation.

There are at least two mechanisms by which polyphenolic compounds could influence NO release: described stimulation of NO synthase activity and preservation and/or stabilization of NO release under basal conditions. The later mechanism includes protection of NO from destruction by superoxides and other free radicals (Schuldt *et al.* 2000, Zavillová *et al.* 2001, Sulová *et al.* 2005). The antioxidant activity of polyphenols in red wine, grape, green and black tea had been documented by their inhibitory effects on human low density lipoprotein oxidation (Frankel *et al.* 1993, Fuhrman *et al.* 1997, Serafini *et al.* 1998). Duthie and Crozier (2000) also reported that most flavonoids are effective antioxidants in a wide range of chemical oxidation systems being capable of scavenging peroxyl radicals, hydroxyl radicals and peroxynitrite. In contrast, Caccetta *et al.* (2000), did not observe an effect on *ex vivo* lipoprotein oxidation despite an increased plasma phenolic concentration after red wine consumption. Provinols™ restored relaxation of the femoral artery to acetylcholine, which was abolished by superoxides. This finding clearly demonstrated that Provinols™ had an *in vitro* antioxidative effect. Moreover, Provinols™ partially affected the concentration-response curve for the NO donor sodium nitroprusside-induced relaxation in rings without endothelium. Both effects were associated with decreased degradation of NO resulting in the improvement of vasorelaxant responses (Zenebe *et al.* 2003). Diebolt *et al.* (2001) also showed that the relaxant effect of red wine polyphenolic compounds involved a mechanism sensitive to superoxide anion scavengers. Schuldt *et al.* (2000) reported that to a limited extent polyphenols were able to scavenge free oxygen radicals resulting in an increase of NO level. However, Andriambeloson *et al*. (1997) showed that red wine polyphenolic compounds at the concentration producing maximal endothelium-dependent relaxation caused no modification of 3-morpholino-sydnonimine concentration -effect curve. The differences in NO donors and polyphenolic substances used may explain the differing vasorelaxant effect in the above experiments.

Recently it was documented that treatment with Provinols™ decreased significantly the tetrachlor-methane-induced endothelemia. The administration of Provinols™ significantly lowered the presence of free endothelial cells circulating in the blood stream. Analogously, the histopathological changes showing cytoplasmic vacuolization and blebbing and detachment of the endothelial cells were dramatically improved when Provinols™ was administered (Janega *et al.* 2005, Babál *et al.* 2006).

Therefore the hypothesis that increased NO pathway contributes to the probable antiarteriosclerotic and antihypertensive effects of Provinols™ was tested by measuring NO synthase activity and structural alterations within cardiovascular tissue (Pecháňová *et al.* 2004a, Bernátová *et al.* 2002).

2. Preventive effect of Provinols™ on blood pressure increase in L-NAME-induced hypertension

Oral administration of Provinols™ was able to produce a decrease in blood pressure in normotensive rats. This hemodynamic effect was associated with enhanced endothelium-dependent relaxation and enhanced expression of inducible NO synthase and cyclooxygenase 2 genes within the arterial wall (Diebolt *et al.* 2001). This effect probably involves NO pathway since Provinols™ is able to produce *ex vivo* endothelium-dependent relaxation, as a result of enhanced NO synthesis as is documented above.

Pecháňová *et al.* (2004a) provided evidence that oral administration of Provinols™ markedly prevented the increase in blood pressure as well as structural and functional cardiovascular changes in the left ventricle and

aorta of rats subjected to chronic inhibition of NO synthesis. Provinols™ reduced myocardial fibrosis, although it did not affect left ventricular hypertrophy. In addition, Provinols™ prevented aortic thickening, attenuated the increase in aortic reactivity to norepinephrine and prevented the decrease in acetylcholine-induced endothelium-dependent relaxation during NO deficiency in rats. These alterations were associated with the increased NO synthase activity, the moderate increase in eNOS expression, and the reduction of oxidative stress, the factors that may be responsible for the beneficial effect of the Provinols™ (Pecháňová *et al.* 2004a).

In general, hypertension is characterized by an increased oxidative stress in various experimental models, including that produced by chronic inhibition of NO synthesis (Usui *et al.* 1999, Kuneš *et al.* 2004). Reduction of hypertension-induced oxidative stress by Provinols™ could account for its beneficial effects. Indeed, polyphenolic compounds contained in red wine have been reported to mediate its antihypertensive effects since the reduction of urinary and plasma values of malondialdehyde (marker of lipid peroxidation and oxidative stress) has been observed in spontaneously hypertensive rats treated with polyphenols (Duarte *et al.* 2001a,b, Duthie *et al.* 1998). We have documented increased oxidative stress in the left ventricle, aorta, brain and kidney of L-NAME-treated rats, resulting in an increase of conjugated dienes concentration (Pecháňová *et al.* 2004a, 2006b). This increase was partially or completely prevented when Provinols™ was given simultaneously with L-NAME. These data strongly suggest that reduced oxidative stress may contribute to the antihypertensive effect of Provinols™ as well as to protection against cardiovascular remodeling in NO-deficient rats.

Chronic Provinols™ treatment alone or in a combination with L-NAME enhanced the activity of NO synthase in the heart, aorta and femoral artery to a level higher than that in the control rats. These data suggest that Provinols™ is also a potent activator of NO synthase activity in the cardiovascular system under *in vivo* conditions (Pecháňová *et al.* 2004a). The moderate increase of eNOS expression by Provinols™ needs not be the only factor responsible for observed high levels of NO synthase activity. Recently, we have reported that the endothelial NO production caused by Provinols™ was associated with the increase in calcium signaling and the activation of tyrosine kinase pathway within the endothelial cells (Martin *et al.* 2002, Zenebe *et al.* 2003). Although the eNOS expression was greater in the left ventricle and aorta from either L-NAME-treated or Provinols™-treated rats, the simultaneous administration of Provinols™ with L-NAME did not produce an additive effect on eNOS expression. It is well accepted that NO by itself is able to elicit the regulation of the activity (Griscavage *et al.* 1995) or expression of eNOS (Shen *et al.* 1999). The possible mechanism of the effect of red wine polyphenols on eNOS expression was recently studied by Wallerath *et al.* (2003). This study provides the evidence that polyphenols of French red wines increased the activity of the eNOS promoter, with the essential trans-stimulated sequence being located in the proximal 326 base pairs of the promoter sequence. The eNOS mRNA stability was also increased by red wine (Wallerath *et al.* 2003). On the other hand, the effect of L-NAME on iNOS expression was different in the left ventricle and aorta. It should be noted that iNOS expression was enhanced threefold in the aorta of L-NAME hypertensive rats, whereas it was suppressed in the left ventricle (Pecháňová *et al.* 2004a). This is in a good agreement with the earlier report by Luvara *et al.* (1998) and with our recent demonstration of aminoguanidine-sensitive NO production in L-NAME hypertensive animals (Pecháňová *et al.* 2004b). The fact that iNOS expression was reduced in Provinols™ plus L-NAME-treated rats suggests that Provinols™-induced restoration of NO production by eNOS prevents the necessity to up-regulate iNOS in these animals.

Previously, we and other workers have reported that chronic inhibition of NO synthesis induced early vascular inflammatory changes as well as subsequent medial thickening, vascular and myocardial fibrosis (Holécyová *et al.* 1996, Babál *et al.* 1997, Tomita *et al.* 1998, Kitamoto *et al.* 2000). Interestingly, we found that Provinols™ treatment partially prevented the increase in myocardial and aortic protein synthesis, aortic thickening as well as myocardial fibrosis produced by chronic inhibition of NO synthesis (Fig. 2 A,B) (Pecháňová *et al.* 2004a). It is possible that these beneficial effects of Provinols™ could refer to the reduced oxidant status and the increased production of NO as indicated by the increase in NO synthase activity in both cardiac and aortic tissues. For the latter, the enhanced NO production could contribute to the anti-inflammatory and anti-remodeling properties of Provinols™ *in vivo*. The activation of NO pathway may be involved in the regulation of production of inflammatory cytokines,

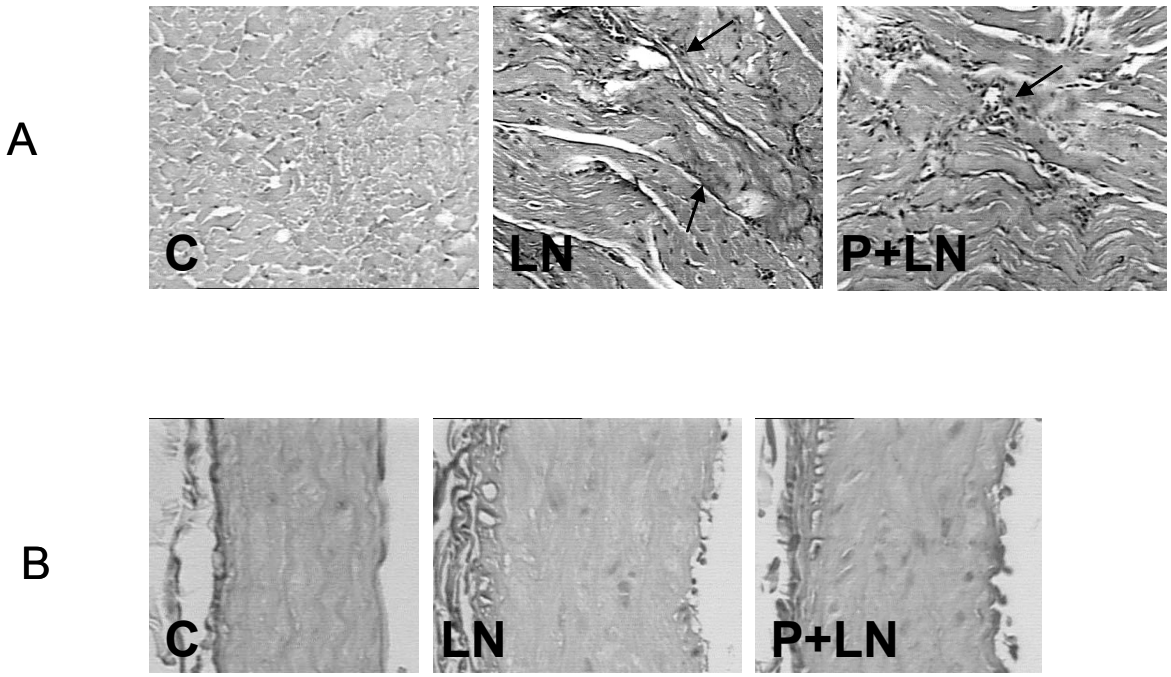

Fig. 2. Chronic administration of N^G-nitro-L-arginine methyl ester (LN) lead to fibrosis development (arrow) that was reduced if Provinols™ was administered simultaneously with LN (P+LN), control (C). Van Gieson, 200 x (A). Chronic administration N^G-nitro-L-arginine methyl ester (LN) lead to significant thickening of the arterial wall when compared to the control (C). The remodeling was significantly reduced if Provinols™ was administered simultaneously with LN (P+LN). Hematoxylin and eosin, 240x (B). (adapted from Pecháňová *et al.* 2004a)

adhesion molecules, and chemokines by inhibition of either transcription nuclear factor-κB (Kitamoto *et al.* 2000) or transforming growth factor-β (Tomita *et al.* 1998). It cannot also be excluded that the reduction of arterial wall thickening by vascular smooth muscle cell proliferation probably involved the downregulation of cyclin A gene expression by red wine polyphenolic compounds (Iijima *et al.* 2000).

Preventive administration of Provinols™ during the development of hypertension also partially protected the renal Na^+,K^+-ATPase molecule against hypertension-induced deterioration *via* increased capability of the enzyme to bind ATP and/or Na^+ as suggested by the decrease of K_m and K_{Na}, respectively, to values even lower than in controls. However, Provinols™ did not prevent the hypertension-induced reduction in the number of active Na^+,K^+-ATPase molecules as shown by similar V_{max} values as compared to the hypertensive L-NAME rats. The above protection is probably mediated by NO-dependent mechanism as suggested by 150 % increase of the NO synthesis in the kidney (Javorková *et al*. 2003, 2004).

3. Effect of Provinols™ during the recovery from established L-NAME-induced hypertension

Bernátová *et al.* (2002) demonstrated that Provinols™ even accelerated the decrease in blood pressure and the improvement of structural and functional cardiovascular changes after the withdrawal of chronic inhibition of NO synthesis. Provinols™ treatment enhanced the regression of aortic wall thickness and improved the decreased endothelium-dependent relaxation in response to acetylcholine and the increased reactivity of the aorta to norepinephrine in hypertension induced by L-NAME. In addition, Provinols™ markedly accelerated the decrease of myocardial fibrosis, although it did not affect the left ventricular hypertrophy. All Provinols™ effects were associated with a decrease in protein synthesis and with an increase of NO synthase activity in the cardiovascular system of rats previously treated with L-NAME (Bernátová *et al.* 2002).

The chronic inhibition of NO synthesis induced by long-term L-NAME treatment in rats results in

hypertension associated with hypertrophy of the left ventricle and cardiac fibrosis as well as with thickening of the arterial media and alteration of vascular functions (Babál *et al.* 1997, Pecháňová *et al.* 1997). With regard to the reduction of blood pressure, the systolic blood pressure of Provinols™-treated rats was not different from that of control rats already after the first week of treatment, whereas two weeks were needed for the recovery group to reach the control value after cessation of L-NAME treatment. Moreover, after three weeks of treatment, Provinols™ induced a more pronounced decrease in blood pressure than that in the recovery group (Bernátová *et al.* 2002). These data suggest that *in vivo* administration of 40 mg/kg Provinols™, used in this study, produced a sufficient concentration of circulating compounds to induce cardiovascular effects. As it was mentioned in the previous part, this dose of Provinols™ leads to a decrease of blood pressure in hypertensive as well as normotensive rats (Diebolt *et al.* 2001). The molecular identity of the polyphenolic compounds responsible for this effect of Provinols™ probably included oligomeric condensed tannins and anthocyanins, both of which were reported to be the red wine polyphenolic compounds that mediate the *ex vivo* relaxation of aortic rings induced by endothelial NO (Andriambeloson *et al.* 1998). The mechanism implicated in the antihypertensive effect of *in vivo* treatment of Provinols™ and other natural dietary polyphenolic compounds is unknown. It has been suggested that the improvement of flow-mediated endothelium-dependent vasodilatation in patients with coronary diseases by ingestion of red grape juice (whose constituents include anthocyanins and tannins) (Stein *et al.* 1999) and the antithrombotic properties (Wollny *et al.* 1999) of red wine *in vivo* in the rat involve NO pathway. However, both the origin of NO production and the mechanism(s) by which it might be enhanced *in vivo* have not been established. We have documented that the accelerated blood pressure decrease in Provinols™-treated rats was associated with an increase of NO synthase activity in the left ventricle and the aorta compared with that of the spontaneous recovery group. In addition, the increase of NO synthase activity occurred sooner in tissues taken from Provinols™-treated rats than in those from the spontaneous recovery group. In the light of these findings, it seems that the effect of Provinols™ on the recovery from hypertension produced by chronic L-NAME treatment is due to enhanced *in vivo* NO production subsequent to increased NO synthase activity in both cardiac and vascular tissues of the rat (Bernátová *et al.* 2002).

As far as the consequences of hypertension are concerned, pressure overload leads to cardiovascular remodeling comprising myocardial and vascular hypertrophy linked to changes of the extracellular matrix compartment. An increased collagen deposition frequently occurs, resulting in fibrosis that is associated with increased myocardial and vascular stiffness and subsequent abnormalities of cardiac and vascular functions (Brilla *et al.* 1990, Doering *et al.* 1988). It has been suggested that a major factor determining the progression of left ventricular hypertrophy to heart failure is the presence of myocardial fibrosis. As reported in our previous studies, chronic inhibition of NO synthesis resulted in hypertrophy of the left ventricle associated with considerable myocardial fibrosis and increased aortic wall thickness (Bernátová *et al.* 2000). Increased protein synthesis in both the left ventricle and the aorta was also observed after L-NAME treatment as was mentioned above. We have found that three weeks after the cessation of L-NAME treatment the reduction of blood pressure toward the control value was associated with normalization of protein synthesis in both the cardiac and aortic tissue without any improvement of cardiac fibrosis, left ventricular hypertrophy, or aortic wall thickness. Thus three weeks of recovery were not sufficient time to observe the full regression of cardiovascular remodeling, although blood pressure returned to normal value. It is noteworthy that Provinols™ treatment reversed cardiovascular remodeling including myocardial fibrosis, enhanced protein synthesis and increased aortic wall thickness produced by chronic inhibition of NO synthesis. The development of vascular remodeling with medial thickening observed in this model of hypertension has been reported to be a consequence of the absence of the anti-inflammatory and anti-arteriosclerotic effects of vascular endothelial NO (Babál *et al.* 1997, Kitamoto *et al.* 2000).

The latter effect occurs *via* the inhibition of the activity of nuclear factor-κB by NO. It is plausible that after Provinols™ treatment, the inhibition of protein synthesis and the decrease in aortic wall thickness result from the increased synthesis of NO. In addition, it cannot be excluded that the inhibition of vascular smooth muscle cell proliferation through the reduction of transcription factor expression might participate in the protective effect of Provinols™. Despite the correction of increased blood

pressure and the increase of NO synthase activity in the spontaneous recovery group, no regression of myocardial fibrosis was observed. In contrast, Provinols™ treatment produced a marked reduction of myocardial fibrosis. It is possible that other unknown mechanisms (unrelated to blood pressure or *in vivo* NO production) contribute to the reduction of myocardial fibrosis by Provinols™. Such mechanisms might include the inhibition of matrix metalloproteinases (Demeule *et al.* 2000), which are involved in maladaptive extracellular protein matrix remodeling leading to myocardial fibrosis. The reduction of blood pressure in Provinols™-treated rats failed to induce the regression of left ventricular hypertrophy despite the reduction of myocardial fibrosis and the inhibition of protein synthesis. It was recently shown that prolonged arterial hypertension may be associated not only with fibrosis but also with an increased volume of interstitial fluid (Laine and Allen 1991). Similarly, L-NAME-induced hypertension is associated with increased permeability of the heart capillaries followed by extracellular microedema as well as mitochondrial edema (Tribulová *et al.* 2000). Thus the lack of Provinols™ effect on left ventricular hypertrophy might be related to its inability to affect interstitial fluid volume in the heart. In addition, it cannot be excluded that a longer period of recovery is needed to achieve reduction of left ventricular hypertrophy, although a decrease of protein synthesis induced by Provinols™, as shown by the reduced [^{14}C]leucine incorporation, might be the first step for the reduction of total heart mass (Bernátová *et al.* 2002).

The reduced endothelium-dependent vasodilatation seen in response to acetylcholine in aortas from L-NAME-treated rats was potentiated in the Provinols™ treated group but not in the spontaneous recovery group (Bernátová *et al.* 2002). A faster and greater increase of NO synthase activity was also found in the aorta from the Provinols™-treated group compared with NO synthase activity in aorta from the spontaneous recovery group. Improved endothelium-dependent vasodilatation is a potential mechanism by which the ingestion of Provinols™ and other red wine polyphenolic compounds may reduce cardiovascular risk. Finally, Provinols™ treatment not only reversed the increased reactivity of the aortas to norepinephrine induced by chronic inhibition of NO synthesis, but it also reduced the norepinephrine-induced contractile response compared to control rats. We found that the treatment with red wine polyphenolic compounds induced the expression of inducible NO synthase and cyclooxygenase-2 genes in the arterial wall from normotensive rats, which together maintain agonist-induced contractility unchanged (Diebolt *et al.* 2001). A similar mechanism might occur in vessels from hypertensive animals. The reason why norepinephrine contractility was reduced in Provinols™-treated rats after the cessation of L-NAME treatment is unknown. It might result from reduced desensitization of smooth muscle guanylyl cyclase to basal release of endothelial NO, from reduced release of vasoconstrictor factors produced by cyclooxygenase or from the enhanced release of other vasodilator factors.

4. Antioxidant effects of Provinols™ on renal tissue

Considerable experimental evidence supports a key role of reactive oxygen species (ROS) in the numerous mechanisms of seemingly unrelated nephropathies (Rodrigo and Bosco 2006). While enzymatic and non-enzymatic systems preserve the antioxidant status, these defense systems become overwhelmed during oxidative stress, which is a metabolic derangement due to an imbalance caused by excessive generation of ROS or a diminished antioxidant capacity. It has long been recognized that ROS are harmful for cells, mainly because they injure lipids, proteins and nucleic acids, leading to structural and functional impairments (Mantle and Preedy 1999). Numerous interventions have been put forward to counteract the effects of ROS by reinforcing the antioxidant defense systems. Dietary supplementation with the antioxidant vitamin E slowed the rate of progression of renal deterioration (Fryer 1997), attenuated the nephrotoxicity caused by ferric nitrilotriacetate (Iqbal and Athar 1998) and ameliorated the glomerulosclerosis occurring in the remnant kidney after subtotal nephrectomy (Pedraza-Chaverri *et al.* 2000). Recently, the possible advantage of a moderate wine consumption in patients with chronic renal failure was suggested (Caimi *et al.* 2004). Therefore, it is expected that the naturally occurring nutritional sources of antioxidants, such as fruits, vegetables, tea or wine, would also attenuate the renal damage caused by oxidative challenges. Polyphenolic compounds, abundant in these nutritional sources, could play a major role in enhancing the antioxidant systems, since they act as ROS scavengers, metal chelators and enzyme modulators (Pietta *et al.* 1998). In agreement with this view, it was

demonstrated that resveratrol, a stilbene polyphenol found in grapes and red wine, suppresses the proteinuria, hypoalbuminemia and hyperlipidemia induced by anti-rat kidney antiserum (Nihei *et al.* 2001). Renoprotective effects have also been reported for other polyphenols such as quercetin (Ishikawa and Kitamura 2000, Sulová *et al.* 2005), alpha-G-rutin (Shimoi *et al.* 1997) and Provinols™ (Rezzani *et al.* 2006). In fact, it has been suggested that Provinols™ (the parts above) prevents the development of hypertension, myocardial fibrosis, aortic thickening, and vascular dysfunction (Bernátová *et al.* 2002, Pecháňová *et al.* 2004a). These effects are associated with the reduction of oxidative stress and increase of the expression of protective genes, such as endothelial nitric oxide synthase.

5. Effect of Provinols™ on Cyclosporine A-induced renal damage

Buffoli *et al.* (2005) showed that the Provinols™ antioxidant capacity was able to minimize renal side effects due to cyclosporine A (CsA) treatment. CsA is the most common immunosuppressive drug used in organ transplantation and autoimmune diseases. There are many clinical and experimental papers showing that its use is often limited by nephrotoxicity. In addition, experimental studies demonstrated that CsA induces alterations both in adult rats and in litters born from treated mothers. Rezzani *et al.* (2004, 2005a) showed that nephrotoxicity was related to tubulointerstitial fibrosis and glomerular vasocon-striction. Tubulointerstitial fibrosis was mainly observed in proximal tubules with respect to distal tubules, suggesting that the proximal tubules were involved in CsA-induced nephrotoxicity. Consequently, the studies, which were performed to reduce renal side effects, demonstrated that Provinols™ is able to counteract these problems. In fact, Provinols™ prevents the increase of systolic blood pressure as well as renal structural and functional injuries in rats treated by CsA (Buffoli *et al.* 2005). Its administration was associated with a decreased tubular injury and interstitial fibrosis and its action was even more pronounced in the glomeruli. Moreover, Provinols™ completely restored the alterations caused by CsA treatment in renal cortex but not in the medulla, in which fibrosis was still observed, especially around the vasa recta. Reduction of both oxidative stress and increased inducible NO synthase expression induced by this drug *via* the nuclear factor-κB pathway may be responsible for the protective effect of Provinols™ on CsA-induced nephrotoxicity. In particular, Provinols™ treatment reduced the increase of iNOS expression in the kidney of CsA-treated rats; this effect probably resulted from the reduction of NF-κB expression. The mechanism by which Provinols™ affects the NF-κB and iNOS pathway remains to be determined, but it acts either by decreasing the level of ROS or by acting on intracellular kinases that alter their expression or activity (Buffoli *et al.* 2005, Rezzani *et al.* 2005b).

Among patients undergoing CsA therapy, increasing numbers of pregnant women have been reported (Bar Oz *et al.* 2001, Tendron-Franzin *et al.* 2004). Because CsA and its active metabolites can cross the placental barrier and enter into fetal circulation (Rezzani *et al.* 1997), its use has been associated with an increased incidence of spontaneous abortion, prematurity and intrauterine growth retardation (Sgro *et al.* 2002). Experimental studies have suggested that in rats CsA induces fetal growth retardation in organ morphogenesis (Rezzani *et al.* 1997) and produced alterations in T-cell maturation with suppression of lymphoproliferative responsiveness to mitogen activation (Padgett and Seelig 2002). In rabbits, CsA is responsible for an impaired nephron differentiation (Papaccio and Esposito, 1990), a permanent nephron deficit inducing systemic hypertension, and progressive renal insufficiency (Tendron *et al.* 2003, Armenti 2004). In particular, Rezzani *et al.* (1997) showed that CsA had deleterious effects on renal organogenesis in litters of CsA-treated mothers and that the combined CsA + Provinols™ treatment is protective against toxic CsA effects in these animals both on cytoarchitecture and on iNOS and MMP2 expression, which are known to play an important role in nephrotoxicity (Rezzani *et al.* 2003, Buffoli *et al.* 2005). In fact, the induction of these proteins after CsA treatment was found and a decrease of their expression after Provinols™ treatment to normal levels was observed. These data suggest that Provinols™ counteract CsA-induced negative effects involving the above-reported proteins as in adult treated animals (Buffoli *et al.* 2005).

Conclusions

The present review provide the evidence that Provinols™ is able to produce *ex vivo* endothelium-dependent relaxation as a result of enhanced NO synthesis. Administration of Provinols™ partially prevents the development of hypertension during NO

deficiency. The effects of Provinols™ include prevention of myocardial fibrosis, reduction of aortic wall thickening and improvement of vascular functions. These functional and structural alterations were associated with significant augmentation of NO production, seen as the increase of NO synthase activity and eNOS expression. Moreover, it has been documented that Provinols™ modulated the oxidative stress in the cardiovascular system during NO deficiency.

Oral administration of Provinols™ induces a faster and more profound decrease of blood pressure in the recovery from hypertension induced by chronic inhibition of NO synthesis. This effect of Provinols™ was associated with a regression in myocardial fibrosis, although it did not reduce L-NAME-induced left ventricular hypertrophy. Most interestingly, Provinols™ treatment reversed the development of aortic wall hyperplasia, improved endothelium-dependent relaxation, and reduced the increased vascular reactivity to vasoconstrictor agonist. Improved endothelium-dependent vasodilatation and endothelium-protective properties are the possible mechanisms, by which the intake of Provinols™ and other red wine polyphenolic compounds may reduce cardiovascular risk. Thus, the beneficial effects of plant polyphenols in prevention of hypertension may result from their complex influence on the NO balance in the cardiovascular system.

Moreover, reduction of oxidative stress and its consequent changes in the renal tissue may be responsible for the beneficial effect of Provinols™ during nephrotoxic alterations.

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