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Research Paper

Resveratrol improves cardiovascular function and reduces oxidative organ damage in the renal, cardiovascular and cerebral tissues of two-kidney, one-clip hypertensive rats

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Abstract

Objectives The putative protective effects of resveratrol against oxidative injury in the heart, kidney and brain tissues of rats induced with the two-kidney, one-clip (2K1C) hypertension model were investigated.

Methods Wistar albino rats were divided into sham-operated ($n = 8$) or 2K1C groups, in which rats received either resveratrol (10 mg/kg per day, i.p., $n = 8$), or saline ($n = 8$) starting at Week 3 after the surgery and continuing for the following 6 weeks. Indirect blood pressure recordings and echocardiographic images were made to evaluate cardiac function. At the end of Week 9 the animals were decapitated and plasma, heart, kidney and brain were taken for biochemical assays, while aortic rings were prepared for vascular reactivity studies.

Key findings 2K1C hypertension resulted in increased blood pressure, aortic hypercontractility and reduced left ventricular function, leading to increased lipid peroxidation and myeloperoxidase activity, concomitant with significant reductions in tissue glutathione, superoxide dismutase, Na^+/K^+-ATPase and catalase activities in the cardiac, renal and brain tissues, indicating the presence of oxidative tissue damage in peripheral target organs. Elevated plasma levels of lactate dehydrogenase, creatine kinase, as well as reduced plasma levels of antioxidant capacity and nitric oxide further verified the severity of oxidative injury. A 6-week treatment with resveratrol reversed all the measured parameters, ameliorated hypertension-induced oxidative injury in the target organs and improved cardiovascular function.

Conclusions Resveratrol improved cardiovascular function through the augmentation of endogenous antioxidants and the inhibition of lipid peroxidation by maintaining a balance in oxidant/antioxidant status, which also ameliorated hypertension-induced oxidative injury in the cardiac, renal and cerebral tissues.

Keywords hypertension; Na^+/K^+-ATPase; oxidative stress; resveratrol

Introduction

Oxidative stress resulting from an imbalance between the generation of reactive oxygen species and the operation of antioxidant mechanisms is important in the pathogenesis of cardiovascular diseases such as atherosclerosis, ischaemic heart disease, heart failure, stroke, hypertension and diabetes. Although the mechanisms responsible for the maintenance of hypertension remain unclear, one hypothesis is that an increase in angiotensin II (Ang II) can activate NAD(P)H oxidase which produces superoxides (O_2^-), modifying the bioavailability of nitric oxide (NO) and increasing vasoconstriction.[1] Experimental evidence shows that increased oxidative stress plays a major role in maintaining high arterial blood pressure and sympathetic drive in a renovascular hypertension model.[2] Recently, it was shown that administration of antioxidant agents, such as vitamins C and E, decreases markers of oxidative stress and improves arterial stiffness and endothelial function in essential hypertensive patients.[3]

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Epidemiological studies suggest that Mediterranean diets including the consumption of wine, particularly of red wine, are associated with reduced risk of cardiovascular disease.[4] The cardioprotective effect of red wine has been attributed to antioxidants present in its polyphenol fraction.[5] Grapes contain a variety of antioxidants, including resveratrol, catechin, epicatechin and proanthocyanidins. Resveratrol, which is present mainly in grape skin, is thought to have diverse antiatherogenic activities,[6,7] including modulation of reactive oxygen species and NO production, and regulation of vascular smooth muscle. The cardiovascular benefits of resveratrol may be due to its direct and indirect antioxidant effects in biological systems.[8,9] Resveratrol may also confer vasculoprotection by regulating the expression of pro-inflammatory and pro-atherogenic genes in endothelial cells.[10] In accordance with these experimental studies, it was shown to exert a cardioprotective effect by lowering plasma lipids and reducing oxidative stress in both pre- and postmenopausal women.[11] Although resveratrol was shown to alleviate oxidative injury in several tissues,[12–14] the possible protective effect of resveratrol on hypertension induced target organ injury has not yet been investigated. In the present study, we aimed to investigate the possible beneficial effects of resveratrol on renovascular hypertension induced oxidative damage in the renal, cardiovascular and cerebral tissues of rats.

Materials and Methods

Animals

All experimental protocols were approved by the Marmara University Animal Care and Use Committee. Male Wistar albino rats (2 months old, 200–250 g) were kept at a constant temperature (22 ± 1°C) with a 12-h light/dark cycle and were fed a standard rat chow.

Surgery and experimental design

Two-kidney, one-clip (2K1C) has been studied as an Ang II-dependent model of renovascular hypertension with elevated circulating levels of Ang II and high Ang II concentration in the cortical tissue of the clipped and non-clipped kidneys.[15] Clipping of the left renal artery and sham surgery were performed as previously described.[16] Briefly, a silver clip (internal diameter 0.25 mm) was placed around the left renal artery of rats ($n = 16$) that were anaesthetized with ketamine (100 mg/kg, i.p.) and chlorpromazine (0.75 mg/kg, i.p.). Half of the group with hypertension was treated intraperitoneally with saline (1 ml/kg per day), while the other half was treated with resveratrol (10 mg/kg per day) starting by the end of Week 3 after the clip placement surgery and continuing for the remaining 6 weeks. The rationale for the selected dose of resveratrol was based on our previous study demonstrating its protective action in other oxidative injury models.[17] In the sham-operated control group ($n = 8$), rats had similar surgical procedures without clip placement.

To obtain basal readings, systolic blood pressure recordings were obtained for all rats before the surgical procedures (clip placement or sham operation), and these measurements were repeated at the end of the Week 3 and Week 9 after surgery. Trunk blood was collected and immediately centrifuged at 3000*g* for 10 min to assay the plasma levels of lactate dehydrogenase (LDH), creatine kinase, antioxidant capacity and NO. Kidney, heart and brain samples were taken and stored at −80°C for the determination of superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and glutathione (GSH) levels, along with myelopreoxidase (MPO) and Na^+/K^+-ATPase activities in these tissues.

Blood pressure measurement

Indirect blood pressure measurement was made by the tail cuff method (Biopac MP35 Systems, Inc. COMMAT Ltd., Ankara, Turkey) before the surgery and at the end of Week 3 and Week 9 after surgery. Initially, the rats were placed for 10 min in a chamber heated to 35°C. Then the rats were placed in individual plastic restrainers and a cuff with a pneumatic pulse sensor was wrapped around their tails. Blood pressure recorded during each measurement period was averaged from at least three consecutive readings on that occasion obtained from each rat.

Echocardiography

Echocardiographic imaging and calculations were done according to the guidelines published by the American Society of Echocardiography[18] using a 12-MHz linear transducer and 5–8-MHz sector transducer (Vivid 3, General Electric Medical Systems Ultrasound, Tirat Carmel, Israel). Under ketamine (50 mg/kg, i.p.) anaesthesia, measurements were made from M-mode and two-dimensional images obtained in the parasternal long and short axes at the level of the papillary muscles after observation of at least six cardiac cycles. Interventricular septal thickness (IVS), left ventricular diameter (LVD) and left ventricular posterior wall thickness (LVPW) were measured during systole (s) and diastole (d). Ejection fraction, fractional shortening and left ventricular mass and relative wall thickness were calculated from the M-mode images using the following formulas: % ejection fraction = $(LVDd)^3 - (LVDs)^3/(LVDd)^3 \times 100$; % fractional shortening = $LVDd - LVDs/LVDd \times 100$; left ventricular mass = $1.04 \times ((LVDd + LVPWd + IVSd)^3 - (LVDd)^3) \times 0.8 + 0.14$; relative wall thickness = $2 \times (LVPWd/LVDd)$.[18]

Vascular reactivity studies

Thoracic aorta were carefully isolated, removed from open-chest animals and placed in a dish containing chilled Krebs-Henseleit buffer solution aerated with 95% O_2 and 5% CO_2. After removal of the surrounding connective tissue, aorta were cut transversely into rings approximately 4-mm wide and mounted in organ baths containing 20 ml of Krebs–Henseleit buffer aerated with 95% O2 and 5% CO_2, maintained at 37°C. The rings were placed under a resting tension of 1.0 g. Following a 60-min period of equilibration, they were exposed to 80 mM KCl.

In the aorta rings, the contractile responses to 10^{-8}–10^{-3} M phenylephrine were determined cumulatively. After a 30-min washout period, the relaxation responses of the same rings were evaluated by adding increasing cumulative concentrations of 10^{-8}–10^{-3} M acetylcholine (ACh), and 10^{-8}–10^{-3} M sodium nitroprusside to rings precontracted with the submaximal dose of phenylephrine (30 μM). Contractile responses to phenylephrine are expressed as percentages of the maximal

contraction induced by 80 mM KCl, and the relaxation responses to ACh are expressed as percentages of the contraction caused by 30 μM phenylephrine. To assess the functional integrity of the endothelium, at the end of the experiments relaxation responses to ACh were obtained in the tissues precontracted with a submaximal dose of phenylephrine. The tissues were excluded if the response was not similar to the previously obtained ones.

Plasma assays

Plasma levels of LDH and creatine kinase were determined spectrophotometrically using an automated analyser (Bayer Opera biochemical analyser; Bayer, Leverkusen, Germany), while total antioxidant capacity was measured by using a colorimetric test system (ImAnOx, Immunodiagnostic AG, Bensheim, Germany), according to the instructions provided by the manufacturer. NO metabolites (nitrates and nitrites) were assayed in plasma by the colorimetric method of Griess after enzymatic conversion of nitrates to nitrites by nitrate reductase using a colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, US).

Measurement of tissue superoxide dismutase and catalase activity

SOD activity in tissue samples was measured according to a previously described method.[19] Briefly, measurements were performed in cuvettes containing 2.8 ml 50 mM potassium phosphate (pH = 7.8) with 0.1 mM EDTA, 0.1 mM 0.39 mM riboflavin in 10 mM potassium phosphate (pH 7.5), 0.1 ml of 6 mM *O*-dianisidin.2 HCl in deionized water, and tissue extract (50, 100 μl). Cuvettes with all their components were illuminated with 20-W Slylvania Gro-Lux fluorescent tubes (Sylvania GRO-LUX F18W/GRO, Erlangen, Germany) that were placed 5 cm above and to one side of cuvettes maintaining a temperature of 37°C. Absorbance was measured at 460 nm with a Schimadzu UV-02 model spectrophotometer (Schimadzu, Tokyo, Japan). A standard curve was prepared routinely with bovine SOD (S-2515-3000 U; Sigma Chemical Co, St Louis, MO, USA) as reference. Absorbance readings were taken at 0 and 8 min of illumination and the net absorbance was calculated.

The method for the measurement of CAT activity is based on the catalytic activity of the enzyme that catalyses the decomposition reaction of H_2O_2 to give H_2O and O_2.[20] Briefly, the absorbance of the tissue samples containing 0.4 ml homogenate and 0.2 ml H_2O_2 was read at 240 nm and 20°C against a blank containing 0.2 ml phosphate buffer and 0.4 ml homogenate for about 1 min.

Measurement of tissue malondialdehyde and glutathione levels

Heart, kidney and brain samples were homogenized with ice-cold 150 mM KCl for the determination of MDA and GSH levels. The MDA levels were assayed for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously.[21] Lipid peroxidation was expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 M/cm and results are expressed as nmol MDA/g tissue. GSH measurements were performed using a modification of the Ellman procedure.[22] Briefly, after centrifugation at 3000*g* for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l $Na_2HPO_4.2H_2O$ solution. A 0.2-ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. GSH levels were calculated using an extinction coefficient of 1.36×10^4 M/cm. Results are expressed in μmol GSH/g tissue.

Measurement of tissue myeloperoxidase activity

Since the activity of MPO, an enzyme that is found predominantly in the azurophilic granules of polymorphonuclear leukocytes, correlates with the histochemically determined polymorphonuclear leukocytes in tissues, MPO activity is frequently utilized to estimate polymorphonuclear leukocyte accumulation in the inflamed tissues. MPO activity was measured in tissues in a procedure similar to that documented by Hillegass *et al*.[23] Tissue samples were homogenized in 50 mM potassium phosphate buffer (pH 6.0), and centrifuged at 41 400*g* (10 min); pellets were suspended in 50 mM potassium phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide. After three freeze and thaw cycles, with sonication between cycles, the samples were centrifuged at 41 400*g* for 10 min. Aliquots (0.3 ml) were added to 2.3 ml of reaction mixture containing 50 mM potassium phosphate buffer, *o*-dianisidine, and 20 mM H_2O_2 solution. One unit of enzyme activity was defined as the amount of MPO present that caused a change in absorbance measured at 460 nm for 3 min. MPO activity was expressed as U/g tissue.

Measurement of Na^+/K^+-ATPase activity

The activity of Na^+/K^+-ATPase, a membrane-bound enzyme required for cellular transport, is very sensitive to free radical reactions and lipid peroxidation. Accordingly, a reduction in Na^+/K^+-ATPase activity indirectly indicates membrane damage. Measurement of Na^+/K^+-ATPase activity is based on the measurement of inorganic phosphate (Pi) released by ATP hydrolysis during incubation of homogenates with an appropriate medium. The total ATPase activity was determined in the presence of 100 mM NaCl, 5 mM KCl, 6 mM $MgCl_2$, 0.1 mM EDTA, 30 mM Tris HCl (pH 7.4), while the Mg^{2+}-ATPase activity was determined in the presence of 1 mM ouabain. The difference between the total and the Mg^{2+}-ATPase activities was taken as a measure of the Na^+/K^+-ATPase activity.[24,25] The reaction was initiated with the addition of the homogenate (0.1 ml) and a 5-min pre-incubation period at 37°C was allowed. Following the addition of 3 mM Na_2ATP and a 10-min re-incubation period, the reaction was terminated by the addition of ice-cold 6% perchloric acid. The mixture was then centrifuged at 3500*g*, and Pi in the supernatant fraction was determined by the method of Fiske and Subarrow.[26] The specific activity of the enzyme was expressed as nmol Pi/mg protein per h. The protein concentration of the supernatant was measured by the method of Lowry *et al*.[27]

Statistical analysis

Statistical analysis was carried out using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA). Each group consisted of eight animals. All data are expressed as

means ± SEM. Groups of data were compared with an analysis of variance followed by Tukey's multiple comparison tests. Values of $P < 0.05$ were regarded as significant.

Results

Blood pressure and echocardiograpy measurements

The basal blood pressure that was recorded before surgery was not different among the groups (Figure 1). In the saline-treated hypertension group, the mean systolic blood pressure was significantly elevated at Week 3 (187 ± 12.4 mmHg; $P < 0.05$) and Week 9 (199 ± 6.8 mmHg; $P < 0.05$) with respect to basal values. In the resveratrol-treated hypertension group, in which the treatments were not yet started at Week 3, mean blood pressure was also elevated (177 ± 6.5 mmHg; $P < 0.05$) at the Week 3 recording. However, systolic blood pressure was reduced significantly after 6 weeks of resveratrol treatment as compared with the saline-treated hypertension group (134 ± 5.6 mmHg; $P < 0.05$).

Table 1 summarizes the transthoracic echocardiograpy measurements of the experimental groups. In the saline-treated hypertension group, left ventricular posterior wall thickness, left ventricular end-diastolic and end-systolic dimensions, as well as relative wall thickness, were increased significantly as compared with sham-operated rats ($P < 0.05$), while the percent fractional shortening and ejection fraction were significantly decreased (Figure 2). On the other hand, in the resveratrol-treated hypertension group, echocardiographic measurements were significantly different compared with those of the saline-treated hypertension group ($P < 0.05$), but similar to those of the sham-operated group.

Vascular reactivity

All vessels were pre-contracted to the same degree with 80 mM KCl and similar tensions were obtained. In the sham-operated control rats, phenylephrine added cumulatively (10^{-8} to 10^{-3} M) caused a concentration-dependent contraction in the aortic rings with a concentration of 6.9×10^{-7} M resulting in a 50% maximal response (Figure 3a). In the saline-treated 2K1C renovascular hypertension group, the contractile responses of the rings to phenylephrine were increased slightly compared with those of the sham-operated group

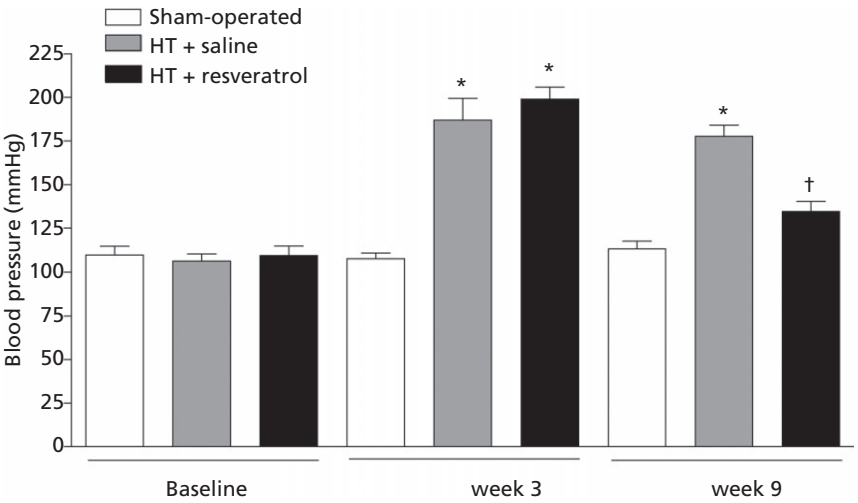

Figure 1 Blood pressure measurements. Systolic blood pressure of the sham-operated, saline-treated hypertension and resveratrol-treated hypertension groups were recorded before the surgery (baseline) and on Week 3 and Week 9 after surgery. *$P < 0.05$, compared with the sham-operated group. †$P < 0.05$, compared with the saline-treated hypertension group.

Table 1 Transthoracic echocardiograpy measurements

	Sham-operated	HT + saline	HT + resveratrol
IVS (mm)	19 ± 0.05	2.2 ± 0.13	2.1 ± 0.12
PW (mm)	1.7 ± 0.04	2.2 ± 0.09*	1.7 ± 0.08†
Relative wall thickness	0.5 ± 0.02	1.0 ± 0.06*	0.7 ± 0.04*†
LVDs (mm)	2.4 ± 0.22	3.3 ± 0.13*	2.7 ± 0.12†
LVDd (mm)	3.8 ± 0.10	5.2 ± 0.15*	4.1 ± 0.16†
Ejection fraction (%)	76.5 ± 2.7	61.8 ± 3.1*	72.5 ± 1.7†
Fractional shortening (%)	39.5 ± 2.6	27.1 ± 2.0*	35.5 ± 2.1†

IVS, interventricular septal thickness; LVDd, left ventricular diameter in diastole; LVDs, left ventricular diameter in systole; PW, left ventricular posterior wall thickness. HT + saline, saline-treated hypertension group; HT + resveratrol, resveratrol-treated hypertension group. Each group consisted of eight animals. *$P < 0.05$, compared with the sham-operated group. †$P < 0.05$, compared with the saline-treated hypertension group.

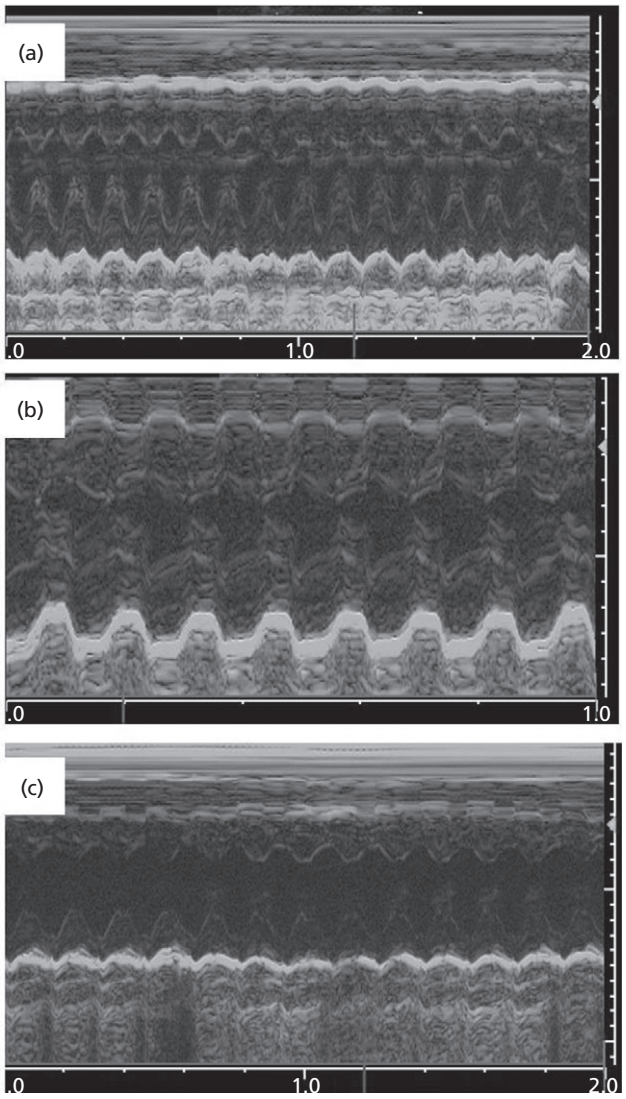

Figure 2 Echocardiograpy measurements. Representative echocardiographic scans of: (a) the sham-operated group with normal M-mode view; (b) the saline-treated hypertension group with increased interventricular septum and left ventricular posterior wall thickness; and (c) the resveratrol-treated hypertension group demonstrating normal M-mode view as the sham-operated group without hypertrophy.

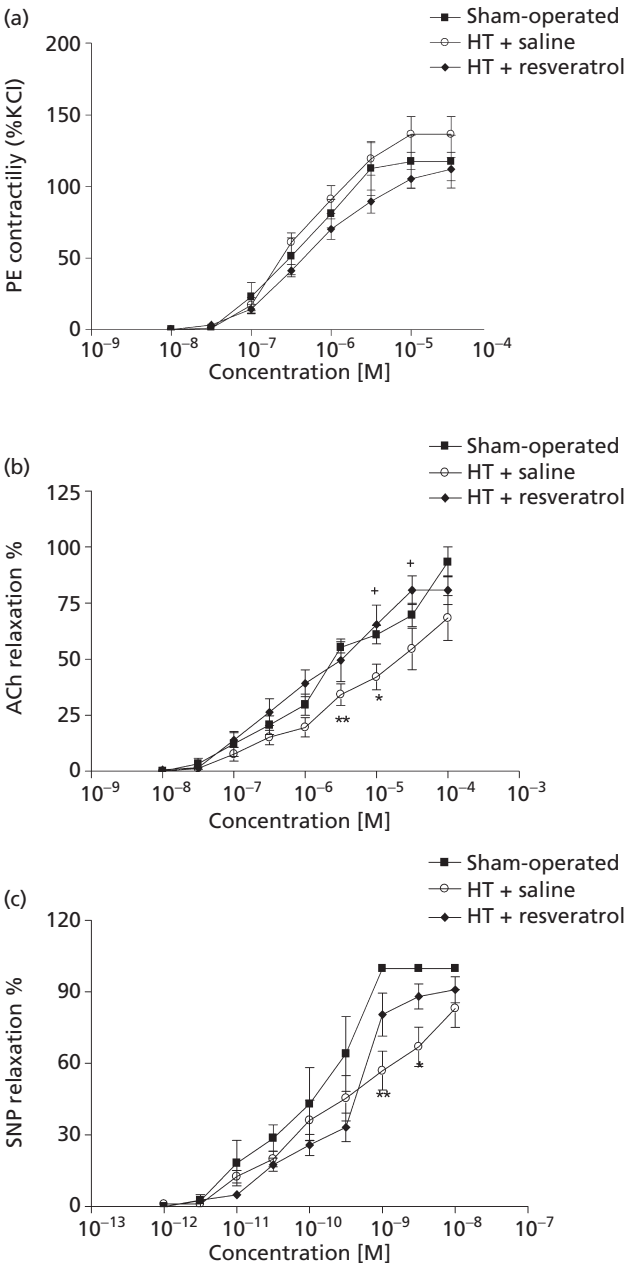

Figure 3 Vascular reactivity. (a) Concentration–response curves obtained by cumulative addition of phenylephrine (PE) to rat aortic rings. (b) Acetylcholine (ACh) concentration–response curves in PE-precontracted rat aortic rings. (c) Sodium nitroprusside (SNP) concentration–response curves in PE-precontracted rat aortic rings. Values are shown as mean ± SEM of 8 experiments. $^{*}P < 0.05$, compared with the sham-operated group. $^{\dagger}P < 0.05$, compared with the saline-treated hypertension group.

($P > 0.05$), requiring a lower concentration to achieve the 50% maximal response to phenylephrine (4.7×10^{-7} M). Treatment of the hypertensive rats with resveratrol improved the contractile responses to phenylephrine (5.7×10^{-7} M).

ACh added cumulatively (10^{-8}–10^{-3} M) to aortic rings precontracted with the submaximal concentration of phenylephrine caused a dose-dependent relaxation response. In the aortic rings of saline-treated hypertensive rats, the relaxation responses to ACh were markedly lower (4.6×10^{-6} M) than those of the sham-operated group ($P < 0.05$) with 2.45×10^{-6} M ED50, while the relaxation responses of the aorta were significantly higher in the resveratrol-treated hypertension group ($P < 0.05$) with 1.4×10^{-6} M ED50 (Figure 3b).

When sodium nitroprusside was added cumulatively (10^{-8}–10^{-3} M) to aortic rings precontracted with the submaximal dose of phenylephrine, dose-dependent relaxation responses were obtained (Figure 3c). Relaxation in response to sodium nitroprusside in aortic rings was not different among the sham-operated, saline-treated hypertension or resveratrol-treated hypertension groups.

Table 2 Biochemical parameters in plasma

	Sham-operated	HT + saline	HT + resveratrol
Lactate dehydrogenase (U/l)	1686 ± 65	2497 ± 170*	1812 ± 130†
Creatine kinase (U/l)	1442 ± 45	1988 ± 81*	1672 ± 73†
Total antioxidant capacity (pg/ml)	419 ± 30.1	217 ± 19.5*	402 ± 29.4†
Nitric oxide (μmol/l)	52.4 ± 3.3	31.9 ± 1.8*	48.7 ± 3.3†

HT + saline, saline-treated hypertension group; HT + resveratrol, resveratrol-treated hypertension group. Each group consisted of eight animals *$P < 0.05$, compared with the sham-operated group. †$P < 0.05$, compared with the saline-treated hypertension group.

Biochemical parameters in plasma

Plasma LDH and creatine kinase levels showed a significant increase in the saline-treated renovascular hypertension group ($P < 0.05$), while resveratrol treatment decreased these elevations ($P < 0.05$, Table 2). In addition, induction of hypertension caused significant decreases in the plasma antioxidant capacity and NO metabolite levels ($P < 0.05$), but these reductions were prevented in the resveratrol-treated hypertensive rats ($P < 0.05$).

Biochemical parameters in tissue

SOD and CAT activities, as well as GSH levels, measured in cardiac, renal and cerebral tissues were reduced in the saline-treated hypertension group as compared with those of the corresponding tissues from the sham-operated control group ($P < 0.05$, Table 3). However, in the resveratrol-treated group, hypertension-induced reductions in these antioxidant parameters were abolished ($P < 0.05$). In accordance with these results, the levels of MDA, which is a major degradation product of lipid peroxidation, were significantly increased in heart, kidney and brain tissues of the saline-treated 2K1C group with respect to the sham-operated control group ($P < 0.05$), while resveratrol treatment caused marked decreases in the MDA levels of all tissues ($P < 0.05$). Similarly, hypertension-induced oxidative stress caused significant increases in the MPO activities of cardiac, renal and cerebral tissue samples when compared with those of the sham-operated control group ($P < 0.05$), indicating increased tissue neutrophil infiltration. However, in hypertensive rats treated with resveratrol, the increase in MPO activity was significantly inhibited ($P < 0.05$). When compared with the sham-operated control group, Na^+/K^+-ATPase activity measured in the studied tissue samples was reduced in the saline-treated renovascular hypertension group, indicating impaired transport function and membrane damage in these tissues ($P < 0.05$). On the other hand, renovascular hypertension-induced reductions in tissue Na^+/K^+-ATPase activity were prevented in the resveratrol-treated hypertension group ($P < 0.05$).

Discussion

In the present study, renovascular hypertension resulted in increased blood pressure and aortic hypercontractility, which was accompanied by reduced left ventricular function. On the other hand, the Ang II-induced hypertension model led to increased lipid peroxidation and MPO activity, concomitant with significant reductions in tissue glutathione, Na^+/K^+-ATPase activity, SOD and CAT activities in cardiac, renal and brain tissues, implicating the presence of oxidative tissue damage. In addition, elevated plasma levels of LDH and creatine kinase, as well as reduced NO and antioxidant capacity levels further verified the severity of oxidative stress. Depending on the isolated vascular strip results and blood pressure measurements, it can be concluded that resveratrol treatment significantly suppressed blood pressure in the 2K1C model, while tissue studies revealed that hypertension-induced oxidative stress was alleviated. Thus, the major conclusions to be drawn from this study were that longterm treatment with resveratrol significantly improved all the measured parameters, attenuated the extent of renovascular hypertension-induced oxidative organ damage and improved cardiovascular function.

Among the experimental models of hypertension, the Ang II-dependent 2K1C model is of potential clinical importance since certain forms of hypertension and congestive heart failure are associated with elevated plasma renin activity and circulating levels of Ang II.[28,29] It was suggested that a compromised mechanism of antioxidant defence and an increase in oxidative damage contribute to the development of hypertension and associated vascular dysfunction in 2K1C rats, while treatment with a SOD-mimetic or NADPH-dependent oxidase inhibitor prevents these effects.[30] Similarly, administration of antioxidants including liposome encapsulated SOD, the permanent SOD-mimetic tempol, vitamins C and E improved endothelium-dependent relaxation, vascular oxidative stress and hypertension in several experimental models of hypertension and in humans.[31,32] Rajagopalan *et al.* have demonstrated that hypertension induced by chronic Ang II infusion is associated with endothelial dysfunction and an increased vascular formation of reactive oxygen species.[33] Sarr *et al.* have also reported that intake of red wine polyphenols prevented hypertension and endothelial dysfunction in rats by blocking vascular NADPH oxidase induction and by preserving arterial NO.[34] The present findings also showed that the 2K1C model caused a significant increase in blood pressure and an impaired aortic contractile activity, while oxidative injury was observed in the studied target tissues. In accordance with previous results, the present study also demonstrated that Ang II-dependent 2K1C hypertension is ameliorated by longterm resveratrol treatment, which appears to act by inhibiting oxidative injury and associated mediators. Moreover, it has been proposed that resveratrol increases the resistance to vascular oxidative stress by scavenging H_2O_2 and preventing oxidative stress-induced endothelial cell death,[35]

Table 3 Biochemical parameters in tissue

Oxidant/antioxidant parameters	Heart			Kidney			Brain		
	Sham-operated	HT + saline	HT + resveratrol	Sham-operated	HT + saline	HT + resveratrol	Sham-operated	HT + saline	HT + resveratrol
Superoxide dismutase (U/mg protein)	1.4 ± 0.1	0.90 ± 0.07*	1.3 ± 0.1†	0.9 ± 0.08	0.6 ± 0.1*	0.9 ± 0.05†	0.8 ± 0.1	0.3 ± 0.05*	0.7 ± 0.1†
Catalase (U/mg protein)	72.0 ± 5.6	42.0 ± 3.7*	63.1 ± 5.6†	56.5 ± 4.1	24.2 ± 4.9*	47.3 ± 5.4†	64.5 ± 4.3	36.8 ± 3.5*	52.0 ± 4.1†
Glutathione (μmol/g)	2.6 ± 0.1	1.3 ± 0.1*	1.8 ± 0.1*†	2.0 ± 0.1	0.9 ± 0.1*	1.3 ± 0.1*†	2.1 ± 0.1	0.95 ± 0.07*	1.5 ± 0.06*†
Malondialdehyde (nmol/g)	20.2 ± 1.1	44.0 ± 2.0*	30.5 ± 1.4*†	25.9 ± 1.7	61.2 ± 3.5*	37.1 ± 2.3*†	8.0 ± 0.7	19.0 ± 1.3*	12.1 ± 1.2*†
Myeloperoxidase (U/g)	8.7 ± 0.6	19.9 ± 1.1*	11.1 ± 0.5*†	5.20 ± 0.4	17.1 ± 1.0*	8.4 ± 0.8*†	5.6 ± 0.6	11.8 ± 0.8*	7.9 ± 0.8*†
Na^{+}, K^{+}-ATPase (μmol/mg protein per h)	2.6 ± 0.3	1.1 ± 0.2*	1.9 ± 0.1†	1.7 ± 0.2	0.7 ± 0.1*	1.2 ± 0.1*†	2.1 ± 0.2	0.8 ± 0.08*	1.8 ± 0.1†

HT + saline, saline-treated hypertension group; HT + resveratrol, resveratrol-treated hypertension group. *$P < 0.05$ compared with the sham-operated group. $^{\dagger}P < 0.05$ compared with the saline-treated hypertension group.

which is suggested to be involved in the vasculoprotection attributed to Mediterranean diets.[36] Despite several studies demonstrating a significant inverse correlation between consumption of antioxidants and cardiovascular risk, the lack of benefit seen in clinical trials to date does not disprove the central role of oxidative stress and the protective effects of antioxidants in atherosclerosis.[37]

In addition to its antihypertensive effect, the aortic ring experiments revealed that resveratrol treatment reduced the aortic hypercontractility of 2K1C hypertensive rats and facilitated ACh-induced relaxation. It was reported that resveratrol elicits vasodilation in large and small vessels[38–40] and induces a concentration-dependent relaxation in human internal mammary artery precontracted with phenylephrine.[41] Endothelial cell culture studies have revealed that resveratrol increases both the expression and activity of endothelial NO synthase,[42] which raises the possibility that in addition to the acute vasodilatory effects, chronic exposure to resveratrol could affect vasomotor function. Chronic resveratrol ingestion at a dose mimicking that obtained from moderate red wine consumption improved ACh-induced, endothelium-dependent vasodilation of arteries in spontaneously hypertensive rats.[43,44] Thus, the present data provide novel evidence of vasorelaxation in renovascular hypertensive animals as a result of chronic resveratrol treatment. We have previously shown that impaired contractile activity of urinary bladder and corpus cavernosum due to chronic nicotine treatment were restored by resveratrol, indicating that resveratrol may provide an important contribution in the prevention of nicotine-induced oxidative damage.[45]

It is well known that stimulation of the renin–angiotensin–aldosterone and sympathetic nervous systems and endothelial dysfunction have an important impact in the pathogenesis of left ventricular hypertrophy.[46] Studies in both humans[40] and animal models[38] have postulated that an increase in oxidative stress could be responsible for hypertension-induced myocardial dysfunction. In agreement with these studies, echocardiographic measurements accomplished on Week 9 of the 2K1C procedure showed the presence of left ventricular dysfunction in hypertensive rats, while the observed oxidative stress suggested that it might be responsible for the associated left ventricular dysfunction. On the other hand, resveratrol treatment decreased blood pressure, depressed hypercontractility and improved cardiac function. Previously, resveratrol treatment was shown to prevent the development of hypertrophy and dysfunction in spontaneously hypertensive rats, while reducing the oxidative stress levels of cardiac tissue.[47] Several studies have suggested that the cardioprotective effects of resveratrol are associated with a reduction in oxidative stress,[11,35,48] which may be partially mediated by its peroxyl radical scavenging activity.[49] Resveratrol and red wine rich in resveratrol enhance the expression of endothelial nitric oxide synthase in human endothelial cells.[50] Accordingly, in the present study, the improvements in cardiac and aortic functions were accompanied with replenished antioxidant status and higher NO levels, verifying the antioxidant and NO-mediated cardioprotective effects of resveratrol.

As evident in many chronic diseases, excessive release of oxygen radicals, if not controlled by the endogenous antioxidant systems, can lead to lipid peroxidation.[51–53] Evidence

obtained from humans with essential hypertension[54] and from animal models[55–57] support the hypothesis that oxidative stress and inflammation are associated with the development of hypertension and hypertension-induced damage in target organs,[58] because oxygen radicals increase renal vascular tone, sensitivity to vasoconstrictors and endothelial dysfunction.[59] On the other hand, blockade of the oxidative stress improves renal blood perfusion and prevents inflammation and fibrosis in the stenotic kidney.[60] In the present 2K1C model, MDA levels in the kidneys, as well as in the cardiac and cerebral tissues, were significantly increased, indicating that oxidative stress generated in the kidneys, which contributes to the generation and maintenance of hypertension, also causes oxidative damage in multiple organs remote to the kidneys, while resveratrol treatment reduced tissue MDA levels and increased plasma NO and antioxidant capacity levels, indicating that hypertension-induced oxidative stress is prevented through the antioxidant properties of this polyphenol. Moreover, decreased activity of Na^+/K^+ ATP-ase, which is a membrane-bound enzyme that requires phospholipids for its activity, also indicates lipid peroxidation-induced injury in the studied tissues.[61] The results demonstrate that treatment with the antioxidant resveratrol reversed the alterations in tissue MDA and GSH levels, SOD, catalase and Na^+/K^+ ATP-ase activities, which are essential for maintaining cell integrity. Thus, in accordance with our previous results,[62,63] the present study demonstrates that resveratrol displays significant beneficial actions against oxidative cellular toxicity in the 2K1C hypertension model.

Since MPO plays a fundamental role in oxidant production by neutrophils and is used as an index for the neutrophil infiltration, the increased MPO activity observed in the present study clearly demonstrates that 2K1C-induced damage in the kidneys and the two target organs investigated is neutrophil dependent. Activated neutrophils release MPO, causing the production of large amounts of HOCl, which oxidizes and damages macromolecules, including proteins, lipids, carbohydrates and nucleic acids. Increasing evidence also suggests that neutrophils release chemotactic substances, which further promote neutrophil migration to the tissue, activate neutrophils and increase the damage.[64] On the other hand, we have previously shown that resveratrol and its derivatives are potent inhibitors of MPO in several inflammatory conditions[62,65,66] Doxorubicin-induced cardiac dysfunction and oxidative tissue injury were reversed by resveratrol via the inhibition of neutrophil infiltration.[18] In parallel with these results, the present data also show that inhibition of neutrophil migration plays an important role in the antioxidant effects of resveratrol in hypertensive rats.

Conclusions

Resveratrol improved cardiovascular function through the augmentation of endogenous antioxidants and the inhibition of lipid peroxidation by maintaining a balance in the oxidant/antioxidant status, which also ameliorated hypertension-induced oxidative injury in cardiac, renal and cerebral tissues. On the basis of these results, further investigation regarding the effects of resveratrol supplementation in experimental and clinical studies is needed to confirm whether it may be beneficial in renovascular hypertension-induced multi-organ dysfunction.

Declarations

Conflict of interest

The authors declare that they have no conflicts of interest to disclose.

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