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The effects of quinapril and atorvastatin on artery structure and function in adult spontaneously hypertensive rats

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Abstract

We studied the combined treatment effects of quinapril and atorvastatin on blood pressure and structure and function of resistance arteries from adult spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto rats (WKY rats). Apoptotic cells were identified by in situ end labeling using the terminal deoxynucleotide transferase-mediated dUTP nick end labeling method. Vascular structure was measured using a morphometric protocol and confocal microscopy and a pressurized artery system was used to study vascular functions. We found that a combined treatment with quinapril and atorvastatin lowered systolic blood pressure in both adult SHR and WKY rats and decreased medial thickness and volume and the number of smooth muscle cell layers in mesenteric arteries, as well as media-to-lumen ratio in the interlobular arteries from SHR but not in those from WKY rats. The number of apoptotic smooth muscle cells was higher in the mesenteric arteries from control WKY rats than control SHR and treatment increased the number of apoptotic smooth muscle cells in the arteries from both SHR and WKY rats. Treatment with quinapril and atorvastatin reduced ventricular weight in SHR and normalized the augmented contractile responses to norepinephrine but did not alter the contraction to electric field stimulation. Relaxation responses to acetylcholine and sodium nitroprusside were not affected by the treatment. We conclude that a combined treatment with quinapril and atorvastatin lowered blood pressure and improved cardiac and vessel hypertrophy and vessel function. An increase in apoptotic smooth muscle cells may be one of the mechanisms underlying the structural improvement.

Keywords: Quinapril; Atorvastatin; Apoptosis; Confocal microscopy; Hypertension; Morphometry; Vascular reactivity

1. Introduction

Both hypertension and hypercholesterolemia frequently occur together (Lemne et al., 1994). Thus, medical treatment in hyperlipidemic patients often includes antihypertensive agents in addition to lipid-lowering treatment. A retrospective analysis on data from controlled clinical trials with fluvastatin and one of the antihypertensives suggests that the efficacy and safety profile of this 3-hydroxy-3-methylglutaryl coenzyme A (HMG–CoA) reductase inhibitor is not affected by concomitant drug treatment with β-blockers, diuretics, calcium antagonists, or angiotensin-converting enzyme inhibitors (Peters et al., 1993). However, it is unknown if HMG–CoA reductase inhibitors would affect the cardiovascular effects of antihypertensive drugs.

Several studies have demonstrated that long-term treatment with an angiotensin-converting enzyme inhibitor can prevent the full development of hypertension in the spontaneously hypertensive rats (SHR) (Díez et al., 1997; Lee et al., 1991). Our results have shown that treatment with quinapril, an angiotensin-converting enzyme inhibitor, significantly lowered systolic blood pressure and ventricular weight in both SHR and normotensive Wistar–Kyoto rats (WKY rats) and improved vessel wall structure by reducing medial thickness and volume in both SHR and WKY rats (Yang et al., 2004). Furthermore, it also

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normalized the augmented contractile response and impaired relaxant response of SHR mesenteric arteries to the level of WKY rats.

There are, however, conflicting reports regarding the effects of HMG–CoA reductase inhibitors on blood pressure and vascular structure and function. Some have reported a blood-pressure-lowering effect and an attenuation of vascular hypertrophy in mesenteric artery and renal arterioles with statin therapy (Lin et al., 1999). Others (Bravo et al., 1998) have suggested that statins may have a detrimental effect on blood pressure and vascular structure (Li et al., 1996). A 30-day treatment of adult SHR with atorvastatin at 50 mg/kg/day reduced the blood pressure by around 20 mm Hg and improved endothelial dysfunction (Wassmann et al., 2001). Previous studies in our lab have shown that a 10-week treatment of SHR with atorvastatin at 0.5, 2 and 5 mg/kg/day caused a slight but significant decrease in blood pressure for 3–4 weeks, followed by an increase in blood pressure, but the blood pressure of WKY rats was not affected (More et al., unpublished results). Treatment with atorvastatin also increased contractile response of the mesenteric arteries to electrical field stimulation and attenuated the response of WKY rats and SHR mesenteric arteries to endothelium-dependent relaxation with acetylcholine.

Vascular remodeling is an important contributor to the pathogenesis of hypertension. Apoptosis of vascular smooth muscle cells has been identified as an essential process involved in the control of vascular remodeling (DeBlois et al., 1997; Bennett, 1999). The exact mechanisms involved in apoptosis, however, remain unknown. Recent reports indicate that certain drugs that are currently used in antihypertensive therapy may exert their effect by promoting smooth muscle cell apoptosis in vivo (DeBlois et al., 1997). We have shown that quinapril increased the number of apoptotic smooth muscle cell in both SHR and WKY rats (Yang et al., 2004), but atorvastatin decreased the number of apoptotic smooth muscle cells in SHR but not in WKY rats (More et al., unpublished results).

The purpose of this study was to determine whether the HMG–CoA reductase inhibitor atorvastatin might interfere with the effects of quinapril on hypertension treatment and on improving vascular structure and function.

2. Methods

2.1. Rat strains and treatment protocol

Male SHR and WKY rats were obtained from the existing colonies at McMaster University's Animal Quarter. Both colonies were derived from the Charles River strains and we have maintained these colonies in our institute by continuous inbreeding. They were kept under a 12 h light : 12 h dark cycle and fed a standard Purina rat diet. Food and water were constantly available. The care of these animals was in accordance with the guidelines of the Canadian Council on Animal Care.

Treatment began when the animals were 15 weeks of age and lasted for 10 weeks. Treatment was given by gavage at 0.5 mg/kg/day of atorvastatin and 10 mg/kg/day of quinapril. Atorvastatin was dissolved in 0.5% methylcellulose and quinapril was dissolved in distilled water. The control groups were given the same volume of 0.5% methylcellulose. Blood pressure was measured using the indirect tail–cuff compression method.

2.2. Apoptosis experiments and confocal microscopy

Perfusion fixation of the mesenteric arteries was conducted as described previously (Dickhout and Lee, 1997; Lee et al., 1983). Mesenteric arteries (the second branch from the superior mesenteric artery) were sampled from the control and treatment groups after the treatment period (25 weeks old) and placed in 4% paraformaldehyde. The arteries were washed in basic physiological salt solution and cut into about 2 mm segments for processing. For apoptotic smooth muscle cell staining, the segments were treated with collagenase and elastase to allow the reagents to reach the smooth muscle cells in the media. Tris buffer (0.1 mol/l, pH 7.4) containing 0.14 mol/l NaCl and 0.01 mol/l $CaCl_2$ was used to dissolve the enzymes. Collagenase (Type 1, Sigma) was added at 300 units/ml and elastase (Type IIA, Sigma) at 30 units/ml. The arteries were incubated in this solution for 1–1.5 h at 37 °C. This digestion step was omitted for apoptotic staining of endothelial cells. For staining of apoptotic cells, an Apoptag Kit was used following a previously described procedure (Dickhout and Lee, 1999). After washing, arterial segments for apoptotic endothelial cell staining were counter-stained with ethidium bromide (20 μg/ml) to allow full morphometry to be performed on the vessels (Dickhout and Lee, 1997). The arteries were mounted in 100% glycerol containing 2.5% 1,4-diazabicyclo [2.2.2] octane (DABCO) (Sigma) as an antifade agent and placed on microscope slides for viewing. A Carl Zeiss LSM 10 System (Carl Zeiss Canada, Don Mills, ON, Canada) was used for confocal microscopy and fluorescence microscopy.

2.3. Morphometry and apoptotic cell quantification

Our dual labeling technique allowed both the determination of artery structure using the ethidium dye and apoptotic cell quantification using the fluorescent probe on the same artery. Apoptotic cell quantification proceeded by optical sectioning traversing through the entire depth of the artery while collecting signal at the 520 nm wavelength (the emittance of the fluorescent probe). Planes within the artery with nuclei emitting in this wavelength were then optically sectioned and the number of nuclei was counted. Morphometric measurement of medial thickness and medial layer area was determined by the Scion Image Program (National Institutes of Health, USA 1997) and medial volume was calculated by using a Cavalierian estimator of volume (Dickhout and Lee, 1997). The cross-sectional area, lumen diameter and the number of smooth muscle cell layers of interlobar, interlobular, and afferent arterioles in the left kidney were measured using a method we have described previously (Dukacz et al., 2001).

2.4. Functional study

The 2nd order branches of the superior mesenteric arteries were isolated with the aid of a dissection microscope. Reactivity studies were carried out using a pressure myograph system (Smeda, 1992).

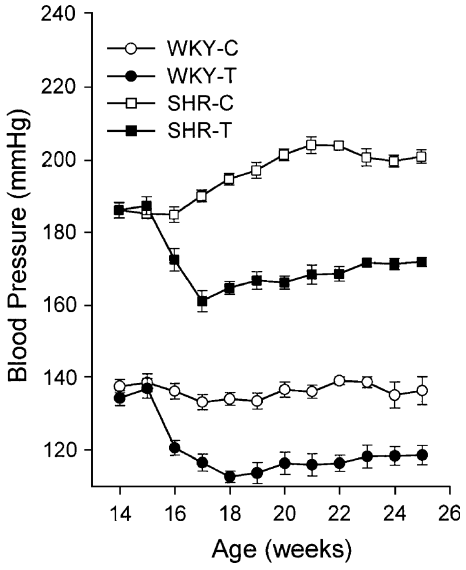

Fig. 1. Systolic blood pressure profile of spontaneously hypertensive rat (SHR) and Wistar–Kyoto rat (WKY rats) (means±S.E.M.) during 10 weeks of combined treatment with quinapril and atorvastatin. The systolic blood pressure of treated groups (T) was significantly lower than that of control groups (C) in both WKY rats and SHR. $n=9$ in control SHR and 8 in control WKY rats; 9 each in treated SHR and WKY rats. Two-way analysis of variance for repeated measures showed that there was a significant difference between the rat strains ($P<0.0001$), between the treatment and control groups ($P<0.0001$) and interaction among the groups and treatment ($P=0.03$).

The bathing solution consisted of the following (mol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.18; KH_2PO_4, 1.08; $NaCO_3$, 25; HEPES, 8; and glucose, 5. The transmural pressure was adjusted to 90 mm Hg for SHR and 60 mm Hg for WKY rats, the optimal pressure of which we have determined for these vessels. The solution in the chamber was continuously bubbled with mixed gas of 95% O_2–5% CO_2, maintained at 37 °C, and was changed every 15–20 min. The transparent image of the vessel under a microscope (model M3C, Leica, Switzerland) was viewed with a video camera and recorded on tape. Changes in lumen diameter were used as indicators of vessel contraction or relaxation.

After 90 min of equilibration, the artery was challenged with 100 mol/l KCl twice at an interval of 30 min. Electrical field stimulation (0.85 ms at 150 V for each pulse, a 10 s train) with increasing frequency (2, 12, and 20 Hz) was applied to the vessel (Dickhout and Lee, 1997) at an interval of 10 min and in the presence of propranolol (1 μmol/l) to block β-receptor-mediated response and cocaine (10 μmol/l) to block neuronal reuptake of neurotransmitter. After 10 min of equilibration in the presence of tetrodotoxin (3 μmol/ml), electrical field stimulation was repeated to determine the nerve origin of the contraction. Tetrodotoxin blocks the sodium channels at the nerve endings. The cumulative concentration–response curve for norepinephrine was also determined in the presence of propranolol and cocaine. Contractile response to each frequency of electrical field stimulation or to each concentration of norepinephrine was expressed as a percentage of the contraction to 100 mmol/l KCl.

To determine the relaxation response, the artery was precontracted with 1 μmol/l norepinephrine in the presence of propranolol and cocaine. Relaxation response to acetylcholine or to sodium nitroprusside was done when the contraction had become stable. Relaxation response was expressed as a percentage of the maximal relaxation produced by 100 μmol/l papaverine at the end of the concentration–response curve study.

2.5. Statistical analyses

All values are expressed as mean±S.E.M. Statistical analyses were performed using SigmaStat Version 2.03 (Jandel Scientific, Corte Madera, CA). For multiple comparisons of at least three groups of data, one-way and two-way analyses of variance and the Tukey multiple comparisons test were used. A two-tailed unpaired Student's *t*-test was used to compare two individual groups. $P \leq 0.05$ was considered significant.

3. Results

3.1. Physical characteristics

After 10 weeks of treatment, systolic blood pressure of treated SHR and WKY rats was significantly less than that of the age-matched control SHR and WKY rats (Fig. 1). In conscious animals, heart rate and body weight values measured weekly from treated SHR and WKY rats were not different from those of controls during the treatment period (data not shown). However, treatment significantly lowered the heart weight in SHR but not in WKY rats (Table 1) but did not affect the wet:dry weight ratio of the heart (Table 1) or kidneys in either SHR or WKY rats (data not shown), indicating the absence of tissue edema.

At 25 weeks of age, plasma cholesterol concentration was significantly higher in the control WKY than control SHR (2.33±0.06 mmol/l versus 1.11±0.04 mmol/l, $P<0.05$). Treatment with atorvastatin reduced its level both in the WKY (1.06±0.11 mmol/l, $P<0.05$ versus control WKY) and SHR (0.88±0.05 mmol/l, $P<0.05$ versus control SHR) (More et al., unpublished results). Similarly at 25 weeks of age, plasma triglyceride concentration was higher in the WKY than SHR (0.80±0.05 versus 0.66±0.07 mmol/l, $P<0.05$). Treatment with atorvastatin reduced its level both in the WKY (0.60±0.04 mmol/l, $P<0.05$ versus control WKY) and SHR (0.28 mmol/l, $P<0.05$ versus control SHR) (More et al., unpublished results).

Table 1
Physical characteristics of control and treated SHR and WKY rats at 25 weeks of age

Parameter	Control SHR ($n=12$)	Treated SHR ($n=8$)	Control WKY ($n=12$)	Treated WKY ($n=13$)
Body weight	380±9	365±7	419±6	408±5
Ventricular wet weight (mg/g body wt)	3.5±0.14	3.0±0.05[a]	2.8±0.03[b]	2.8±0.05[b]
Ventricular dry weight (mg/g body wt)	0.80±0.04	0.70±0.01[a]	0.64±0.01[b]	0.64±0.02[b]
Ventricular wet:dry weight ratio	4.4±0.13	4.3±0.05	4.4±0.05	4.3±0.03

Values are mean±S.E.M. [a]$P<0.05$ when compared with control SHR; [b]$P<0.001$ when compared with control SHR.

Table 2
Mesenteric artery structure parameters from control and treated SHR and WKY rats at 25 weeks of age

Parameter	Control SHR ($n=9$)	Treated SHR ($n=9$)	Control WKY ($n=8$)	Treated WKY ($n=9$)
Lumen diameter (μm)	265.7±23.0	254.9±27.0	315.9±17.6	304.8±35.3
Medial volume ($\mu m^3/\mu m$)	21,630±1259	18,281±238[a]	18,165±839[a]	16,357±1230[a]
Medial thickness (μm)	33.3±1.4	27.6±1.2[a]	24.6±0.7[a]	23.8±1.3[a]
Medial SMC layers	4.1±0.2	3.4±0.1[a]	2.9±0.2[a]	2.9±0.1[a]

Values are mean±SEM. [a]$P<0.05$ when compared with control SHR.

3.2. Morphometry

Values from morphometric measurements of perfusion-fixed mesenteric arteries at maximal relaxation showed no significant difference in lumen size among control and treated SHR and WKY rats (Table 2). Values from control SHR medial wall thickness and smooth muscle cell layers and medial volume were significantly larger than control WKY rats. Treatment with quinapril and atorvastatin resulted in a persistent reduction of these parameters in the SHR arteries but not in those from the WKY rats (Table 2). In the renal vascular bed, values on media-to-lumen ratio, medial thickness, wall to lumen ratio, and the number of smooth muscle cell layers were significantly higher in the control SHR than control WKY rats in the interlobular artery (Table 3). Treatment with quinapril and atorvastatin resulted in a persistent reduction of media-to-lumen ratio but did not affect the number of smooth muscle cell layers in the SHR. Such a treatment did not affect the vessel wall parameters in the WKY rats. There were no significant differences in the vessel wall parameters between control SHR and WKY rats in the afferent arterioles and interlobar arteries between control SHR and WKY rats and treatment had no effects on these arteries (data not shown).

3.3. Apoptosis

Apoptotic endothelial cells were clearly visible in arteries that did not undergo enzyme digestion (Fig. 2A), whereas apoptotic smooth muscle cells were present in arteries that were treated with the enzymes (Fig. 2B). In arteries treated with DNase I, all the cells in the medial and intimal layers showed fluorescent labeling (Fig. 2C). This indicated that the fluorescent probe was able to penetrate fully the blood vessel wall. The number of apoptotic endothelial cells in the mesenteric arteries from control SHR and control WKY rats was similar and treatment did not change this (Table 4). In contrast, the number of apoptotic smooth muscle cells in control WKY rats was significantly higher than control SHR. Treatment significantly increased the number of apoptotic smooth muscle cells in the arteries from both SHR and WKY rats.

3.4. Functional changes

Electrical field stimulation caused a frequency-related contraction of the mesenteric arteries from SHR and WKY rats (Fig. 3A). Incubation of the arteries with tetrototoxin or phentolamine abolished these responses (data not shown). In the control groups the magnitudes of contraction to higher frequencies of electrical field stimulation (12 and 20 Hz) were markedly greater in SHR than in WKY rats. Treatment with quinapril and atorvastatin did not alter electrical field stimulation-induced contraction response.

Norepinephrine induced a concentration-related contraction of the mesenteric arteries from SHR and WKY rats ranging in concentration from 3×10^{-9} to 10^{-4} mol/l (Fig. 3B). In the control groups the contraction produced by the higher concentrations of norepinephrine (3×10^{6}–3×10^{5} mol/l) were higher in SHR than in WKY rats. Treatment with quinapril and atorvastatin reduced the exaggerated response to norepinephrine to the level of control WKY rats. The response to norepinephrine in WKY rats was not affected by the treatment.

Relaxation induced by acetylcholine and sodium nitroprusside was comparable in arteries from control SHR and WKY rats. Treatment with quinapril and atorvastatin did not alter the responses to acetylcholine and sodium nitroprusside (data not shown).

4. Discussion

The major findings from the present study were that combined treatment with quinapril and atorvastatin 1) decreased systolic blood pressure in both SHR and WKY rats; 2) reduced medial thickness, medial volume and smooth muscle cell layers in mesenteric arteries, as well as media-to-lumen ratio in the interlobular arteries from SHR but not in WKY rats; 3) increased the number of apoptosis in smooth muscle cells in SHR and WKY; and 4) normalized the augmented contractile responses to norepinephrine in SHR mesenteric arteries to the level of

Table 3
Morphometric analysis of interlobular arteries from the kidney of control and treated SHR and WKY rats at 25 weeks of age

Parameter	Control SHR ($n=10$)	Treated SHR ($n=9$)	Control WKY ($n=10$)	Treated WKY ($n=9$)
Lumen area (5×10^3 μm^2)	5.28±1.01	4.33±0.54	7.68±2.22	7.96±1.19
Media area (5×10^3 μm^2)	12.38±1.01	10.63±1.12	9.99±1.32	12.70±1.23
Media-to-lumen ratio	3.93±0.30	2.90±0.22[a]	2.47±0.32[a]	2.05±0.28[a]
Lumen diameter (μm)	66.92±6.63	65.46±4.19	80.10±10.01	95.84±6.87
Medial thickness (μm)	66.89±2.46	61.31±3.52	53.03±3.41[a]	67.17±5.67
Wall-to-lumen ratio	1.25±0.08	1.00±0.06[a]	0.81±0.07[a]	0.76±0.09[a]
Medial SMC layers	2.96±0.02	2.72±0.09	2.48±0.09[a]	2.38±0.13[a]

Values are mean±SEM. [a]$P<0.05$ when compared with control SHR.

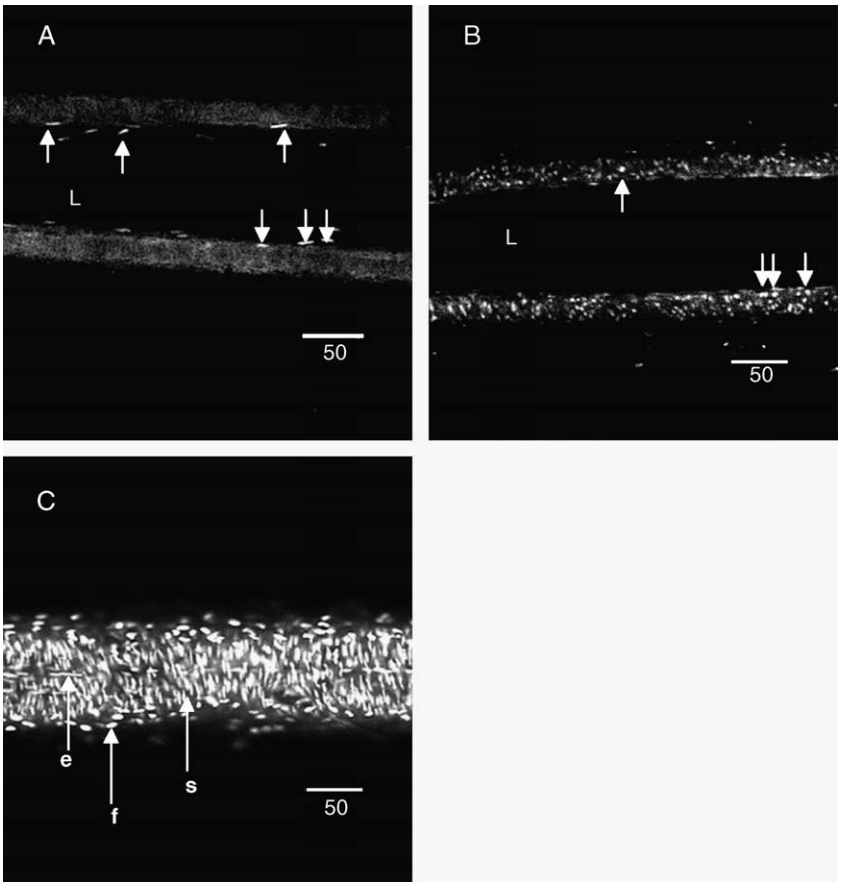

Fig. 2. (A) Optical section showing the presence of apoptotic cells (arrows) on the endothelial surface of a control WKY rat vessel without treatment with digestive enzymes. (B) A control SHR artery with enzyme treatment showing labeling of apoptotic smooth muscle cells in the medial wall (arrows). (C) Optical section of an artery from a control WKY rat treated with DNase I (positive control) showing fluorescent labeling of all cell nuclei in the adventitial, medial and intimal layers (C). e, endothelial cell nuclei; s, smooth muscle nuclei; f, fibroblast nuclei of the adventitial layer; and L, lumen of the artery. Magnification bar=50 μm.

WKY rats. These changes may be due to the effects of quinapril on the vessel structure and functions as discussed below.

In this study, we found that chronic combined treatment with quinapril and atorvastatin still caused a significant decrease in blood pressure in SHR and WKY rats, although previous result in our laboratory showed that treatment with atorvastatin for 10 weeks caused a slight elevation of blood pressure when treatment was initiated in young adult SHR. This indicated that quinapril exerted a dominant blood pressure control effect during the concomitant treatment. The principal mechanism of blood pressure reduction by angiotensin-converting enzyme inhibitors is multi-faceted, which includes a decrease angiotensin II formation and bradykinin degradation.

In humans with hypertension and in various animal models of hypertension, hypertension is associated with hypertrophy of heart and resistance vessels. We have found in this study that the cardiac weight, medial thickness, media-to-lumen ratio, medial volume and smooth muscle cells layers of mesenteric resistance arteries, as well as media-to-lumen ratio of interlobular arteries from SHR were greater than that from age-matched WKY rats. This is consistent with findings from others studies (Dickhout and Lee, 1997; Lundie et al., 1997). Several studies in animal models and in humans with hypertension have shown that angiotensin-converting enzyme inhibitors are very effective in reversing the cardiac and vascular structural change, yielding an increase in cardiac and vascular compliance (Brilla et al., 1996; Yang et al., 2004). Our findings of

Table 4
Number of apoptotic endothelial cells and smooth muscle cells from the mesenteric arteries of control and treated SHR and WKY rats at 25 weeks of age

Cell type	Control SHR ($n=9$)	Treated SHR ($n=9$)	Control WKY ($n=8$)	Treated WKY ($n=9$)
Endothelial cells (# apoptotic cell/1×10^5 μm^2)	2.72±1.14	3.13±1.31	1.38±0.64	1.24±0.69
Smooth muscle cells (# apoptotic cell/3×10^4 μm^3)	6.34±1.80	17.45±2.20[a]	11.95±2.98[a]	16.66±3.39[b]

Values are mean±SEM. [a]$P<0.05$ when compared with control SHR; [b]$P<0.05$ when treated WKY rats were compared with control WKY rats.

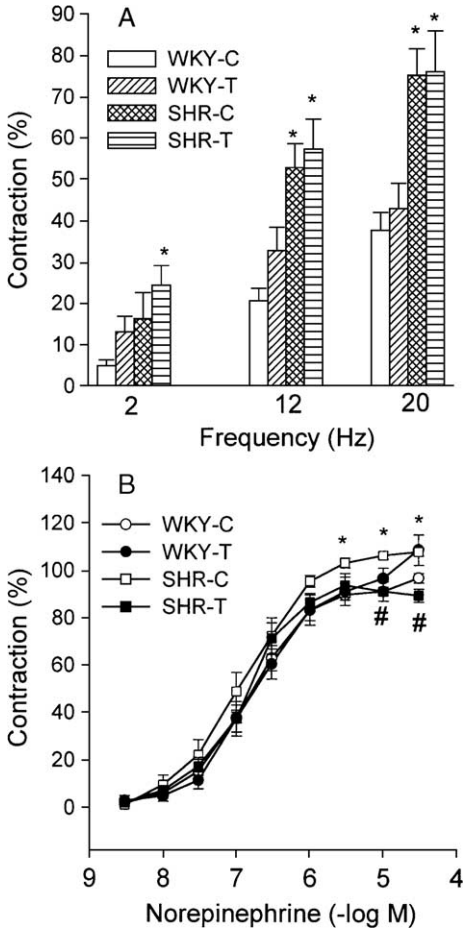

Fig. 3. (A) Contractile responses of mesenteric arteries to electrical field stimulation. Contraction to electrical field stimulation is expressed as a percentage of contraction to 100 mM KCl. *$P<0.05$ versus respective WKY rats. $n=9$ in control SHR, 8 in control WKY rats; 9 each in treated SHR and WKY rats. (B) Concentration–response curve of mesenteric arteries to norepinephrine. Contraction to electrical field stimulation is expressed as a percentage of contraction to 100 mM KCl. *$P<0.05$ versus WKY control rats; $^{\#}P<0.05$ versus SHR control. $n=9$ in control SHR, 8 in control WKY rats; 9 each in treated SHR and WKY rats.

decreased cardiac weight, medial thickness, media-to-lumen ratio, medial volume and smooth muscle cell layers of mesenteric resistance arteries, as well as media-to-lumen ratio of interlobular arteries from SHR, are in support of these findings. We also found that these parameters were not affected by concomitant treatment with atorvastatin. It is possible that the vascular effects we have seen in this study was due mainly to the effects of quinapril because these results were similar to our results when only quinapril was used (Yang et al., 2004).

The potential biological processes involved in remodeling are many, including an increase in the length of vascular smooth muscle cells (Dickhout and Lee, 2000). However, apoptosis of vascular smooth muscle cells is also a major determinant of vascular smooth muscle cells number in remodeling. Previous studies from our laboratory have found that there was a reduced incidence of apoptosis in the medial smooth muscle cells of SHR as compared with WKY rats at 1–2 weeks of age before the onset of vessel wall hypertrophy (Dickhout and Lee, 1999). We had postulated that the reduced incidence of apoptosis might contribute to the development of vessel wall hypertrophy found in these animals at 4 weeks of age, thereby contributing to the development of hypertension. Our present study suggests that the reduced incidence of apoptosis in SHR may continue into adulthood and may therefore also be involved in the maintenance of vessel wall hypertrophy and hypertension. Antihypertensive therapy in SHR with losartan or enalapril increased the rate of smooth muscle cell apoptosis which resulted in medial mass reduction and smooth muscle cell number reduction in SHR aorta (DeBlois et al., 1997), which is a large conducting vessel. Furthermore, antihypertensive treatment of SHR with quinapril increased the expression of Bax protein, a known apoptosis inducer, in the intramyocardial arteries (Díez et al., 1997), but this is an indirect method to detect apoptosis. In adult SHR where we had carried out a time-dependent study on the change in the number of apoptotic smooth muscle cells due to quinapril treatment (Yang et al., 2004), we found that this increase in the number of apoptotic smooth muscle cells was already present 1 week after the initiation of the treatment and a change in medial volume was found at 3 weeks. A similar number of apoptotic smooth muscle cells was found at 3, 5, and 10 weeks. However, there was no further reduction in medial volume after 5 and 10 weeks of treatment, suggesting that the loss of smooth muscle cells due to apoptosis was compensated probably by smooth muscle cell proliferation or hypertrophy, or both, to maintain a steady state of medial volume. Our results are probably the first to provide direct evidence that an angiotensin-converting enzyme inhibitor such as quinapril can still exert its effect by increasing the incidence of medial smooth muscle cell apoptosis in the resistance mesenteric arteries from both SHR and WKY rats, when it is used in conjunction with a statin.

The reason for the increased incidence of apoptosis in smooth muscle cells is unknown. Many factors are known to influence the rate of apoptosis in various cell types. Pollman et al. (1996) have shown that nitric oxide induces vascular smooth muscle cell apoptosis by a cGMP-dependant pathway, whereas angiotensin II inhibits apoptosis by stimulating AT_1 receptor. They speculated that the balance between nitric oxide and angiotensin II may play an important role in determining vessel wall cellularity and thereby modulate vascular structure. Therefore it is possible that apoptosis due to a combined treatment with quinapril and atorvastatin was due to an increased production of nitric oxide and a reduced production of angiotensin II. An enhanced production of nitric oxide and an up-regulation of inducible nitric oxide synthase, as has been shown with enalapril in a balloon arterial injury model (Ohwada et al., 2002), may also be involved. However, the fact that a combined treatment with quinapril and atorvastatin did not

alter the response of the arteries to acetylcholine probably indicates that nitric oxide may not be involved.

Chronic treatment with quinapril and atorvastatin normalized the exaggerated contractile responses to norepinephrine in SHR mesenteric arteries. We have previously found that treatment with quinapril normalized the exaggerated contractile responses to electrical field stimulation and to norepinephrine in SHR mesenteric arteries (Yang et al., 2004), but treatment with atorvastatin increased the contractile responses to electrical field stimulation in SHR (More et al., unpublished data). Therefore the normalization of the contractile response to norepinephrine in SHR due to the combined treatment should be the effect of quinapril. This is consistent with a number of other studies which showed that angiotensin-converting enzyme inhibitors attenuated vascular reactivity in the SHR (Cline, 1985). The normalization of the exaggerated response of SHR mesenteric arteries to electrical field stimulation and norepinephrine due to a combined treatment may be due to a reduction of the medial smooth muscle cell mass and therefore a reduction in the reactivity of the vessel to sympathetic stimulation.

In summary, we have shown in this study that a combined treatment with quinapril and atorvastatin decreased the systolic blood pressure of both SHR and WKY rats and reduced cardiac weight and vascular remodeling in the resistance arteries from the mesenteric bed and interlobular arteries. This remodeling in vessel wall was also correlated with a reduction in the reactivity of the arteries and an increase in the number of apoptotic smooth muscle cells in the mesenteric arteries.

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