BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2009), 158, 541–547

0007-1188/09
www.brjpharmacol.org

RESEARCH PAPER

Chronic treatment with pravastatin prevents early cardiovascular changes in spontaneously hypertensive rats

M Kassan, MJ Montero and MA Sevilla

Departamento de Fisiología y Farmacología, Facultad de Farmacia, Universidad de Salamanca, Salamanca, Spain

Background and purpose: This study investigates the effect of pravastatin on blood pressure, cardiovascular remodelling and impaired endothelial function induced as early signs of cardiovascular disease in young spontaneously hypertensive rats (SHR).
Experimental approach: Eight-week-old SHR were treated for 4 weeks with pravastatin (20 $mg \cdot kg^{-1} \cdot day^{-1}$). Systolic blood pressure was measured periodically during the study using the tail-cuff method. At the end of the study, the left ventricular weight /body weight ratio was used as an index of left ventricular hypertrophy (LVH). Vascular function, superoxide ($O_2^{-\cdot}$) production and structure were studied in aortic rings. Lipid peroxidation was measured in plasma (thiobarbituric acid reactive substances assay).
Key results: Systolic blood pressure was lower in treated SHR than in control SHR, at the end of the study (171 ± 1 vs. 159 ± 2 mmHg, $P < 0.05$), and LVH was significantly reduced by pravastatin (2.7 ± 0.02 vs. 2.5 ± 0.01 $mg \cdot g^{-1}$, $P < 0.05$). Vascular responses to sodium nitroprusside and phenylephrine were similar in both groups; nevertheless, the relaxation response to acetylcholine was higher in the treated rats (45.6 ± 2.6 vs. 58.1 ± 3.2 %, $P < 0.05$). Vascular $O_2^{-\cdot}$ and plasma thiobarbituric acid reactive substances were reduced by pravastatin treatment, and urinary nitrites was elevated. Finally aortic wall became thinner after pravastatin treatment.
Conclusions and implications: Chronic treatment with pravastatin attenuated the increase of systolic blood pressure in SHR, prevented early LVH and improved vascular structure and function. These effects were accompanied by decreased measures of oxidative stress and improvements in NO production.
British Journal of Pharmacology (2009) **158,** 541–547; doi:10.1111/j.1476-5381.2009.00339.x; published online 23 July 2009

Keywords: pravastatin; hypertension; ventricular hypertrophy; endothelial dysfunction; oxidative stress

Abbreviations: LVH, left ventricular hypertrophy; SHR, spontaneously hypertensive rats; SBP, systolic blood pressure; TBARS, thiobarbituric acid reactive substances

Introduction

There is accumulating evidence that the statins [3-hydroxy-3methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] exert numerous beneficial effects that are apparently independent of their action on blood lipids; these include effects on the cardiovascular system, kidneys, bones and glucose metabolism (McFarlane *et al.*, 2002).

A number of clinical trials have shown that statins significantly reduce cardiovascular morbidity and mortality. Potential mechanisms that may mediate beneficial cardiovascular action of statins include modulation of endothelium function (Laufs *et al.*, 1998; Alvarez de Sotomayor *et al.*, 2000; Mital *et al.*, 2000; Mehta *et al.*, 2001), anti-inflammatory action (Egashira *et al.*, 2000), antioxidant properties (Yamamoto *et al.*, 1998; Zhou *et al.*, 2008), plaque stabilization (Crisby *et al.*, 2001), effects on thrombosis (Rosenson and Tangney, 1998) and vasculogenesis (Vasa *et al.*, 2001). Moreover, although statins share a common lipid-lowering effect, there are differences within this class of drugs not only in their lipid-lowering potential but also in their non-lipid effects (Chong *et al.*, 2001). These potential differences necessitate careful and systematic studies involving each member of the statin family.

Direct effects of statins on vascular cells could have important implications for the development of endothelial dysfunction (Wassmann *et al.*, 2001). Disruption of the delicate balance of the NO system and especially the vascular production of reactive species (ROS) promote the development of

Correspondence: Dr María Angeles Sevilla, Laboratorio de Farmacognosia y Farmacología, Facultad de Farmacia, Campus Miguel de Unamuno, 37007 Salamanca, Spain. E-mail: masevilla@usal.es
Received 3 December 2008; revised 2 February 2009; accepted 1 March 2009

endothelial dysfunction. In this context, it has been reported that, for example, the NO and endothelium system may be directly influenced by statins (Hernandez-Perera *et al*., 1998; Laufs *et al*., 1998).

In the present study we examined cardiovascular effects of pravastatin, an HMG-CoA reductase inhibitor. Experiments were developed to investigate the effects of this statin on blood pressure, endothelial dysfunction and vascular ROS in conductance arteries of young spontaneously hypertensive rats (SHR) at an early stage of cardiovascular disease. As plasma lipid levels are normally low in rats and statins usually do not modify lipid profile in rats, they provide an excellent model for studying the other cardiovascular effects of statins.

Methods

All procedures were carried out in accordance with conventional guidelines for experimentation with animals. Eight-week-old male SHR rats were used (Janvier, France). They were housed four per cage in a regulated environment with a 12 h light/dark cycle in a standard experimental laboratory of the Animal Experimentation Service of the Salamanca University. The animals had free access to food and water.

Animals were randomized to receive during 4 weeks: (i) tap water (control), and (ii) pravastatin 20 $mg \cdot kg^{-1} \cdot day^{-1}$ (P-20) dissolved in drinking water. The concentration was adjusted for the daily water intake and body weight.

Systolic blood pressure (SBP) was measured in awake rats with an automated multi-channel system, using the tail-cuff method with a photoelectric sensor (Niprem 546, Cibertec SA, Madrid, Spain) as previously described (Sevilla *et al*., 2004). SBP was measured before starting the study and every week during the treatment. After 4 weeks animals were placed in metabolic cages, and urine samples were collected to determine the nitrate/nitrite excretion. Blood samples were taken to determinate plasma concentrations of total cholesterol and the levels of lipid peroxidation.

Rats were anaesthetized (pentobarbital, 60 $mg \cdot kg^{-1}$, i.p.), exsanginated, and tissues were removed immediately. The heart and the thoracic aorta were removed from the animal and placed in chilled Krebs solution of the following composition (in mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 11, pH 7.4.

Aorta preparation and mounting

The thoracic aorta was carefully cleaned of fat and connective tissue and cut into rings (3 mm in length) that were placed between stainless steel hooks and set up in organ chambers filled with 5 mL of Krebs solution, gassed with carbogen (95% O_2 and 5% CO_2) and kept at 37°C. One of the hooks was fixed to the bath and the other connected to an isometric force transducer (UF1, Harvard apparatus Inc., USA). Force was recorded on a PC computer using Lab Chart version 3.4 software and a Power Lab/800 data acquisition system (AD Instruments, Chalgrove, UK). All rings were allowed to equilibrate for 1 h at a resting tension of 2 g. The Krebs solution was changed every 30 min, and tension was reset to 2 g during this period. Then the vessels were exposed to phenylephrine (1 μM), and the presence of functional endothelium was assessed by the ability of acetylcholine (ACh, 1 μM) to induce relaxation. After a washout period, cumulative concentration response curves of phenylephrine (0.01–100 μM) were obtained. After precontraction with phenylephrine (1 μM) and a steady maximal contraction, cumulative concentration response curves were obtained for ACh (0.01–10 μM) or sodium nitroprusside (SNP, 0.01–10 μM). Each curve was obtained in a different ring.

Histomorphology of aorta

Samples were processed as previously described (Guerrero *et al*., 2006). In brief, aortic samples were fixed, dehydrated and embedded in paraffin. Sections (4 μm thick) were stained with haematoxylin–eosin and examined under light microscopy with an attached video camera. Using an image software (Image J, http://rsb.info.nih.gov/ij/), the internal and external perimeters of the medial layer were measured and converted into internal and external radii (R_i and R_e respectively) according to the formula: perimeter = $2[\pi]R$, where $2 \times R_i$ is the internal diameter (L) and $R_e - R_i$ is the wall thickness (W). Medial cross-sectional area (CSA_m) was calculated as: $CSA_m = [\pi](R_e^2 - R_i^2)$.

Detection of superoxide anion

Production of superoxide anion (O_2^{-}) was assessed by lucigenin-enhanced chemiluminescence assay. Briefly, segments of thoracic aorta were incubated in HEPES-buffer (in mM: NaCl 119, HEPES 20, $MgSO_4$ 1, KCl 4.6, KH_2PO_4 0.4, Na_2HPO_4 0.15, $NaHCO_3$ 5, $CaCl_2$ 1.2, glucose 5.5, pH 7,4) gassed with carbogen and maintained at 37°C for 30 min. Then samples were transferred into tubes containing 1 mL of HEPES-buffer with lucigenin (5 μM). Lucigenin chemiluminescence was then recorded every 30 s for 5 min in a luminometer (LUMAT LB-9507, Berthold Technologies, Bad Wildbad, Germany). Basal and reduced nicotinamide adenosine dinucleotide phosphate (NADPH, 100 μM)-stimulated production were measured and expressed as relative luminescence units $(RLU) \cdot min^{-1} \cdot mg^{-1}$of dry tissue.

Nitrite measurements

Nitrite concentration was determined in urine by a modification of Griess reaction as described previously (Valdivielso *et al*., 2001). Briefly, 500 μL of sample were mixed with 250 μL of Griess reagent (1% sulphanilamide, and 0.1% naphthyl ethylenediamine dihydrochloride, in 2.5% orthophosphoric acid) and incubated for 15 min at room temperature. Absorbance was measured at 560 nm. Calibration was carried out using sodium nitrite.

Cholesterol measurements

The total cholesterol was measured in plasma samples by using a cholesterol assay kit (Biosystems S.A., Barcelona, Spain) following the manufacturer's indications. The enzymatic procedure involves cleavage of the cholesterol esters by cholesterol esterase and oxidation of the free cholesterol by

cholesterol oxidase. These reactions may be quantified photometrically by use of hydrogen peroxide-dependent colour-forming
reactions.

Left ventricular hypertrophy
The heart was removed and placed immediately in Krebs solution at 37°C to remove excess blood. The atrium was removed, and all the epicardial fat was scraped off. The right and the left ventricle were separated, regarding the interventricular septum as an integral part of the left ventricle, and this portion was weighed. Left ventricular hypertrophy index (LVH) was calculated by using left ventricular weight/body weight ratio.

Lipid peroxidation
Plasma levels of thiobarbituric acid reactive substances (TBARS) were determined as an index of lipid peroxidation, following the method described by Ohkawa *et al.* (1979) and modified by us. An aliquot of 250 μL of plasma was added to 1 mL of a reaction mixture containing thiobarbituric acid (37%), trichloroacetic acid (15%) and hydrochloric acid (11.32 N), then samples were warmed at 90°C for 30 min and centrifuged. The absorbance of the supernatants was measured at 535 nm. Data are expressed as concentration of TBARS (μM).

Data analysis
Data are expressed as mean ± SEM. The responses to ACh and SNP are expressed as percentages of phenylephrine contraction. EC_{50} values indicate the concentration of each agonist producing 50% of the maximal response and were calculated by using the GraphPad Prism 4.0 computer programme (GraphPad, San Diego, CA, USA) and expressed as negative log molar concentration (pD_2). Statistical calculations for significant differences were performed by using Student's *t*-test or two-way ANOVA as appropriate. Significance was accepted at $P < 0.05$.

Materials
The drugs used were: phenylephrine hydrochloride, ACh chloride, SNP, N,N-dimethyl-9,9-biacridinium dinitrate (lucigenin) and NADPH tetrasodium salt, haematoxylin and eosin, all purchased from Sigma Chemical Co., USA. Pravastatin was generously supplied by Menarini S.A. (Firenze, Italy).

Stock solution of drugs were made up in ultra-pure water and stored at –20°C, and appropriate dilutions were made on the day of the experiment. Drug and molecular target nomenclature conforms to the British Journal of Pharmacology Guide to Receptors and Channels (Alexander *et al.*, 2008).

Results

Systolic blood pressure
Before treatment, SBP was similar and pathologically elevated in both groups. Throughout the study we saw a gradual increase only in control group. Therefore a significant reduction in blood pressure was observed in the P-20 group as shown in Figure 1.

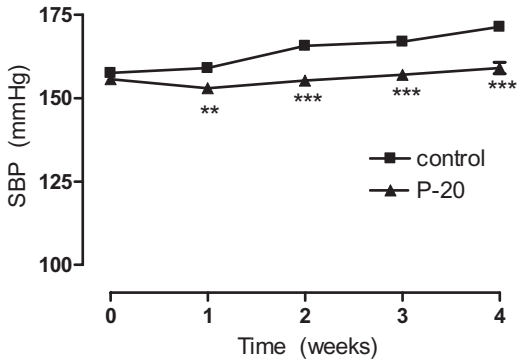

Figure 1 Systolic blood pressure (SBP) in untreated (control) and pravastatin-treated (20 $mg \cdot kg^{-1} \cdot day^{-1}$) (P-20) spontaneously hypertensive rats. Values are means ± SEM ($n = 12$ per group). $^{**}P < 0.01$ and $^{***}P < 0.001$ different from control.

Table 1 Body weight, cardiac morphology and plasma cholesterol

	Control	*P-20*
BW, g ($n = 12$)	298 ± 6	296 ± 7
LVW, mg ($n = 12$)	805 ± 18	767 ± 19**
LVH, $mg \cdot g^{-1}$ ($n = 12$)	2.70 ± 0.02	2.59 ± 0.02**
Cholesterol, $mg \cdot L^{-1}$ ($n = 5–6$)	933 ± 129	921 ± 71

BW, body weight; LVH, left ventricular hypertrophy index; LVW, left ventricle weight.
Control, untreated spontaneously hypertensive rats; P-20, treated spontaneously hypertensive rats.
Values are means ± SEM. $^{**}P < 0.01$ versus control.

Body weights were similar in both groups at the end of the study. Pravastatin reduced significantly the left ventricular weight, and this lead to an improvement of the LVH index. Plasma total cholesterol levels were not altered by the treatment (Table 1).

Thoracic aorta rings
The vasorelaxation to increasing concentrations of ACh and SNP is shown in Figure 2A and B. Whereas the endothelial-independent response to SNP was not altered by the treatment with pravastatin, this statin markedly increased the ACh-induced vasodilatation. The maximum response (E_{max}) to ACh in the untreated (control) group was less than 50% (Table 2), which suggested the presence of endothelial dysfunction that pravastatin was able to improve.

The contractor response to phenylephrine of the aortas from control and treated rats was similar in both groups (Figure 2C). E_{max} and pD_2 values are shown in Table 2. Responses to KCl (80 mM) were not altered by pravastatin treatment (data not shown).

Histomorphology of aorta
Pravastatin induced a marked reduction in wall thickness and cross-sectional area with a significantly increased lumen. This

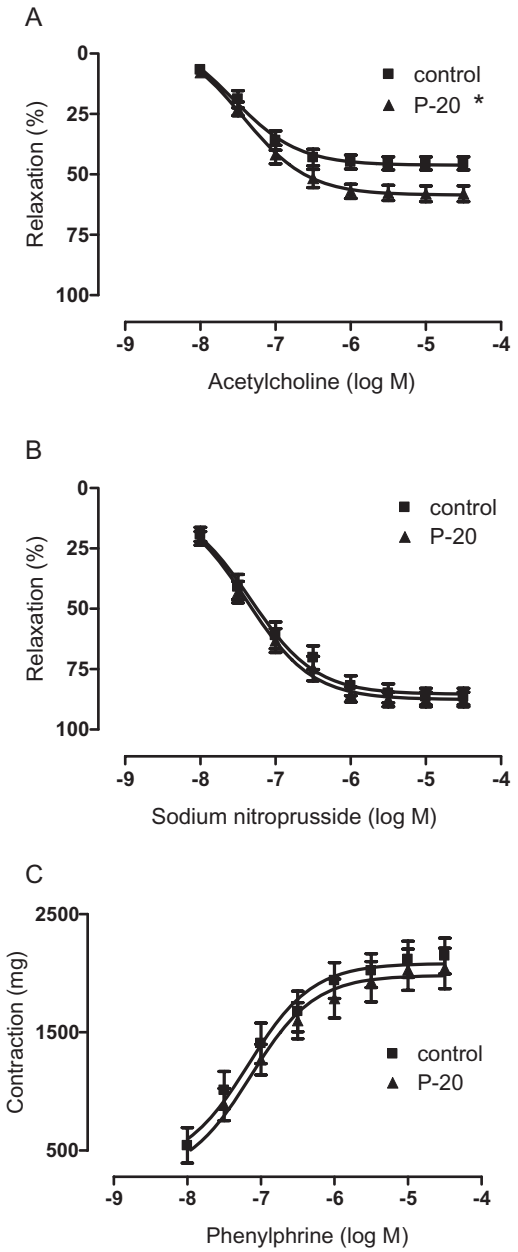

Figure 2 Concentration–response curves to (A) acetylcholine, (B) sodium nitroprusside and (C) phenylephrine in aortic rings isolated from untreated (control) and pravastatin-treated (20 mg·kg^{-1}·day^{-1}) (P-20) spontaneously hypertensive rats. *P < 0.05 different from control.

led to higher values in the wall/lumen (W/L) ratio in untreated SHR, compared with pravastatin-treated rats. (Table 3)

Detection of superoxide anion

The treatment with pravastatin in absence of NADPH caused a slight reduction of superoxide production although the difference between groups did not reach statistical differences (control, 49.4 ± 4.7 and P-20, 39.5 ± 4.3 RLU·min^{-1}·mg^{-1}, P = 0.07). When experiments were carried out in presence of NADPH, we observed a significant reduction in luminescence values in aortic rings from the pravastatin-treated group (Figure 3).

Nitrite measurements

The nitrite concentrations of treated and untreated SHR measured in urine are shown in Figure 4. Pravastatin increased significantly the levels of nitrite found in urine.

Lipid peroxidation

Plasma TBARS levels taken as an index of lipid peroxidation were lower in treated SHR than control SHR at 12 weeks of age (Figure 5).

Discussion

Our findings indicate that 20 mg·kg^{-1}·day^{-1} of pravastatin attenuated the increase of SBP in SHR, reduced the production of ROS and improved endothelium dysfunction and wall remodelling in aorta. In addition, this treatment prevented cardiac hypertrophy, reduced circulating markers of lipid peroxidation (TBARS) and up-regulated eNOS production. Moreover these beneficial cardiovascular effects exerted by pravastatin were independent of its action on cholesterol levels.

The current observation that the chemiluminescence is smaller in resting as well as stimulated rings from treated rats indicated that tissue production of superoxide anions by NADPH oxidase were reduced by pravastatin. Several studies indicate that the prevention of superoxide production in endothelial (Wagner *et al.*, 2000) and smooth muscle cells (Wassmann *et al.*, 2002) by statins could be linked to Rac

Table 2 Parameters of concentration–response curves in aorta rings

	ACh		*SNP*		*PE*	
	pD_2	E_{max} *(%)*	pD_2	E_{max} *(%)*	pD_2	E_{max} *(mg)*
Control (n = 7–10)	7.56 ± 0.17	45.56 ± 2.66	7.33 ± 0.13	86.45 ± 3.40	7.16 ± 0.20	2174 ± 151
P-20 (n = 9–12)	7.43 ± 0.13	58.11 ± 3.27*	7.38 ± 0.12	87.56 ± 3.01	7.13 ± 0.19	2041 ± 171

E_{max} to acetylcholine (ACh) and sodium nitroprusside (SNP) are expressed as percentage relaxation of contractions evoked by phenylphrine (PE, 10^{-6} M), and E_{max} to PE is expressed as milligrams; pD_2 values are calculated as the negative log molar of the agonist inducing a half-maximal response.
Control, untreated spontaneously hypertensive rats; P-20, treated spontaneously hypertensive rats.
Values are means ± SEM. *P < 0.05 versus control.

Table 3 Parameters of thoracic aorta morphometry

	W (μm)	CSA_m (mm^2)	L(μm)	W/L
Control ($n = 6$)	119.8 ± 2.4	0.56 ± 0.01	1419 ± 8	0.084 ± 0.001
P-20 ($n = 6$)	93.4 ± 1.5***	0.49 ± 0.08***	1553 ± 8***	0.060 ± 0.001***

CSA_m, cross-sectional area; *L*, lumen; *W*, wall thickness; *W*/*L*, wall thickness to lumen ratio.
Control, untreated spontaneously hypertensive rats; P-20, treated spontaneously hypertensive rats.
Values are means ± SEM. ***$P < 0.001$ versus control.

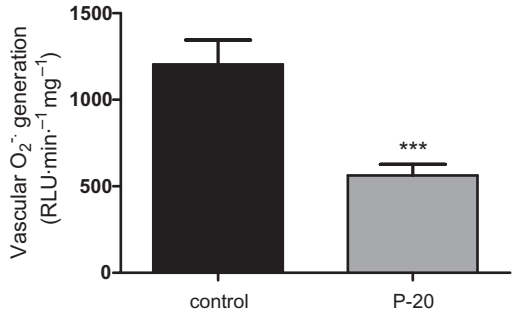

Figure 3 Vascular superoxide anion $O_2^{\cdot-}$ production after stimulation with nicotinamide adenosine dinucleotide phosphate (100 μM) in untreated (control) and pravastatin-treated (20 mg·kg^{-1}·day^{-1}) (P-20) spontaneously hypertensive rats. Values are means ± SEM (n = 8 per group). ***$P < 0.001$ different from control.

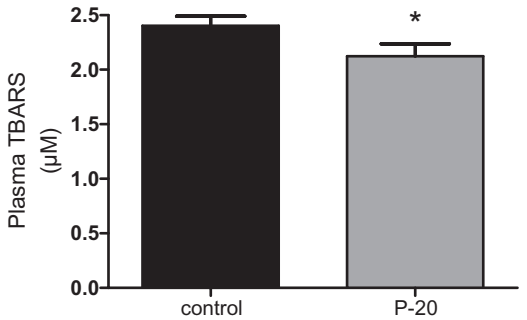

Figure 5 Effect of pravastatin on plasma thiobarbituric acid reactive substances (TBARS) levels in untreated (control) and pravastatin-treated (20 mg·kg^{-1}·day^{-1}) (P-20) spontaneously hypertensive rats. Values are means ± SEM (n = 5 per group). *$P < 0.05$ different from control.

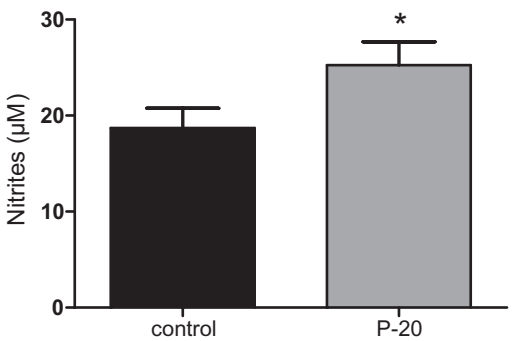

Figure 4 Concentration of nitrites in urine of untreated (control) and pravastatin-treated (20 mg·kg^{-1}·day^{-1}) (P-20) spontaneously hypertensive rats. Values are means ± SEM (n = 5 per group). *$P < 0.05$ different from control.

translocation that is required for NADPH oxidase activation. On the other hand, chronic treatment with antioxidants drugs down-regulates the expression of this enzyme (Oelze *et al.*, 2006; Sánchez *et al.*, 2006) and could be another possibility to explain our results as pravastatin has antioxidant properties. Further studies would be necessary to decide between these mechanisms.

While the response to SNP in aorta was unaltered, we saw that the relaxation to ACh was improved in treated SHR, compared with the control group. These results are in accordance with similar studies using atorvastatin, which reported up-regulation of vascular eNOS expression and enhancement of eNOS activity (Wassmann *et al.*, 2001). The improvement in responses to ACh could be explained by this mechanism that may result in increased production of NO that contributes to the improvement of endothelium dysfunction. The increased level of nitrites in urine observed by us in rats treated with pravastatin supports this hypothesis. On the other hand, uncoupling of eNOS results in increased formation of oxygen radicals by NOS and reduced NO production *in vitro* (Werner *et al.*, 1995; Reif *et al.*, 1999). Shinozaki *et al.* (2007) report an inhibition of uncoupled eNOS-dependent $O_2^{\cdot-}$ production by pitavastatin in insulin-resistant rats and propose that enhancement of eNOS activity *in vivo* is one of the initial and essential steps for the observed beneficial effects of statin treatment. Moreover, $O_2^{\cdot-}$ leads to the formation of hydroxyl radicals, which may be cytotoxic to endothelial cells by direct peroxidation of either lipids or proteins (Freeman and Crapo, 1982). Animals treated with pravastatin showed levels of TBARS in plasma lower than untreated SHR, indicating a reduction of lipid peroxidation. We know that this method has been frequently criticized as too unspecific and prone to artefacts during sample workup, but it still can give us some estimate of the MDA levels when a treated group is compared with a control group under the same conditions.

The SHR strain is a model of chronic essential hypertension, and their blood pressure rises from 4 weeks old. At this time, minor differences in LVH are observed, with respect to Wistar Kyoto (WKY) rats but these are increased progressively until animals are 20 weeks old (Adams *et al.*, 1989). Our study was performed when hypertrophy is being developed and comparing untreated SHR with pravastatin-treated SHR, we observed a significant reduction in LVH. In our opinion, the mechanisms by which pravastatin prevents cardiac hypertrophy is probably not only due to its antihypertensive effect, as that was minor. It has been reported that cardiac endothelin (ET-1) levels were significantly higher at 8 weeks of age in SHR compared with WKY rats (Iyer *et al.*, 1995), and this could be important in triggering cardiac hypertrophy (Iemitsu *et al.*,

2001). Results from Lee *et al*. (2005) suggested a crucial role for the cardiac endothelin system in the early development of ventricular hypertrophy in SHR. They found that prevention of hypertrophy by pravastatin was associated with low ET-1 levels and, furthermore, such effects were specifically prevented by mevalonic acid. Statins also prevent the synthesis of isoprenoid intermediates of the cholesterol biosynthesis pathway, such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP), which serve as important lipid attachments for the post-translational modification of several proteins (Takemoto and Liao, 2001). Because Rho is a major target of GGPP, inhibition of Rho and its downstream target, Rho kinase, is a likely mechanism to be mediating some of the pleiotropic effects of statins on cardiovascular disease (Laufs *et al*., 2000). Recently, in a murine model of angiotensin II-induced hypertension, pravastatin attenuated the increase of Rho kinase improving hypertrophy and fibrosis of the heart (Xu *et al*., 2008). Simvastatin also reverses cardiac hypertrophy caused by disruption of the bradykinin-2 receptor (Osorio *et al*., 2008).

Studies made by our group in SHR and WKY confirm that a hypertensive state induces structural modifications in conductance arteries. Hypertension leads to thickening of arterial wall and increased lumen (Guerrero *et al*., 2006). In this study pravastatin treatment led to significant reduction of wall thickness and cross-sectional area in aorta indicating a beneficial effect in remodelling that could contribute to its antihypertensive effect.

There is an imbalance between growth and apoptosis of vascular smooth muscle cells (VSMC) during vascular remodelling development that pravastatin could reverse. The mevalonate pathway plays a role in cell growth and, particularly, this pathway yields a series of isoprenoids that are vital for the post-translational isoprenylation of proteins like Ras, involved in growth and differentiation of VSMC (Yang *et al*., 2001), and Rho involved in apoptosis (Guijarro *et al*., 1999). Short-term use of fluvastatin in rabbits inhibited proliferation of VSMC in the media and migration to the intima and induced apoptosis (Ye *et al*., 2000). In addition, the elevated urinary excretion of nitrites and decreased production of superoxide that we observed after pravastatin treatment improved endothelial function and could contribute to prevent remodelling.

In conclusion, our results demonstrate that pravastatin, independent of its lipid-lowering properties, could be a useful therapeutic agent to prevent the development of cardiovascular disorders in pre-hypertensive state.

Acknowledgements

The authors like to thank the Menarini laboratory for the generous supply of pravastatin, to A Morales PhD for his kind support in the nitrite determination and to T Hernández for excellent technical assistance in histological studies.

Conflict of interest

The authors state no conflict of interest.

References

Adams MA, Bobik A, Korner PI (1989). Differential development of vascular and cardiac hypertrophy in genetic hypertension. Relation to sympathetic function. *Hypertension* **14**: 191–202.

Alexander SP, Mathie A, Peters JA (2008). Guide to receptors and channels (GRAC), 3rd edn. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Alvarez de Sotomayor M, Herrera MD, Marhuenda E, Andriantsitohaina R (2000). Characterization of endothelial factors involved in the vasodilatory effect of simvastatin in aorta and small mesenteric artery of the rat. *Br J Pharmacol* **131**: 1179–1187.

Chong PH, Seeger JD, Franklin C (2001). Clinically relevant differences between statins: implication for therapeutic selection. *Am J Med* **111**: 390–400.

Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J (2001). Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. *Circulation* **103**: 926–933.

Egashira K, Ni W, Inoue S, Kataoka C, Kitamoto S, Koyanagi M *et al*. (2000). Pravastatin attenuates cardiovascular inflammatory and proliferative changes in a rat model of chronic inhibition of nitric oxide synthesis by its cholesterol-lowering independent action. *Hypertens Res* **23**: 353–358.

Freeman BA, Crapo JD (1982). Biology of disease: free radicals and tissue injury. *Lab Invest* **47**: 412–425.

Guerrero EI, Ardanaz N, Sevilla MA, Arévalo MA, Montero MJ (2006). Cardiovascular effects of nebivolol in spontaneously hypertensive rats persist after treatment withdrawal. *J Hypertens* **24**: 151–158.

Guijarro C, Blanco-Colio LM, Massy ZA, O'Donnell MP, Kasiske BL, Keane WF *et al*. (1999). Lipophilic statins induce apoptosis of human vascular smooth muscle cells. *Kidney Int* **71** (Suppl. l): S88–S91.

Hernandez-Perera O, Perez-Sala D, Navarro-Antolin J, Sanchez-Pascuala R, Hernandez G, Diaz C *et al*. (1998). Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J Clin Invest* **101**: 2711–2719.

Iemitsu M, Miyauchi T, Maeda S, Sakai S, Kobayashi T, Fujii N *et al*. (2001). Physiological and pathological cardiac hypertrophy induces different molecular phenotypes in the rats. *Am J Physiol Regul Integr Comp Physiol* **281**: R2029–R2036.

Iyer RS, Singh G, Rebello S, Roy S, Bhat R, Vidyasagar D *et al*. (1995). Changes in the concentration of endothelin-1 during development of hypertensive rats. *Pharmacology* **51**: 96–104.

Laufs U, La Fata V, Plutzky J, Liao JK (1998). Upregulation of endothelial nitric oxide synthase by HMG CoA reductase inhibitors. *Circulation* **97**: 1129–1135.

Laufs U, Endres M, Stagliano N, Amin-Hanjani S, Chui DS, Yang SX *et al*. (2000). Neuroprotection mediated by changes in the endothelial and cytosketketon. *J Clin Invest* **106**: 15–24.

Lee TM, Lin MS, Chou TF, Tsai CH, Chang NC (2005). Effect of pravastatin on development of left ventricular hypertrophy in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* **289**: H220–H227.

McFarlane SI, Muniyappa R, Francisco R, Sowers JR (2002). Pleiotropic effects of statins: lipid reduction and beyond. *J Clin Endocrinol Metab* **87**: 1451–1458.

Mehta JL, Li DY, Chen HJ, Joseph J, Romeo F (2001). Inhibition of LOX-1 by statins may relate to upregulation of eNOS. *Biochem Biophys Res Commun* **289**: 857–861.

Mital S, Zhang X, Zhao G, Bernstein RD, Smith CJ, Fulton DL *et al*. (2000). Simvastatin upregulates coronary vascular endothelial nitric oxide production in conscious dogs. *Am J Physiol Heart Circ Physiol* **279**: H2649–H2657.

Oelze M, Daiber A, Brandes RP, Hortmann M, Wenzel P, Hink U *et al*.

(2006). Nebivolol inhibits superoxide formation by NADPH oxidase and endothelial dysfunction in angiotensin II-treated rats. *Hypertension* **48**: 677–684.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* **95**: 351–358.

Osorio JC, Cheema FH, Martens TP, Mahmut N, Kinnear C, Gonzalez AM *et al.* (2008). Simvastatin reverses cardiac hypertrophy caused by disruption of the bradykinin 2 receptor. *Can J Physiol Pharmacol* **86**: 633–642.

Reif A, Fröhlich LG, Kotsonis P, Frey A, Bömmel HM, Wink DA *et al.* (1999). Tetrahydrobiopterin inhibts monomerization and is consumed during catalysis in neuronal NO synthase. *J Biol Chem* **274**: 24921–24929.

Rosenson RS, Tangney CC (1998). Antiatherothrombotic properties of statins: implications for cardiovascular event reduction. *JAMA* **279**: 1643–1650.

Sánchez M, Galisteo M, Vera R, Villar IC, Zarzuelo A, Tamargo J *et al.* (2006). Quercetin downregulates NADPH oxidase, increases eNOS activity and prevents endothelial dysfunction in spontaneously hypertensive rats. *J Hypertens* **24**: 75–84.

Sevilla MA, Voces F, Carrón R, Guerrero EI, Ardanaz N, San Román L *et al.* (2004). Amlodipine decreases fibrosis and cardiac hypertrophy in spontaneously hypertensive rats: persistent effects after withdrawal. *Life Sci* **75**: 881–891.

Shinozaki K, Nishio Y, Ayajiki K, Yoshida Y, Masada M, Kashiwagi A *et al.* (2007). Pitavastatin restores vascular dysfunction in insulin-resistant state by inhibiting NAD(P)H oxidase activity and uncoupled endothelial nitric oxide synthase-dependent superoxide production. *J Cardiovasc Pharmacol* **49**: 122–130.

Takemoto M, Liao JK (2001). Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors. *Arterioscler Thromb Vasc Biol* **21**: 1712–1719.

Valdivielso JM, Crespo C, Alonso JR, Martínez-Salgado C, Eleno N, Arévalo M *et al.* (2001). Renal ischemia in the rat stimulates nitric oxide synthesis. *Am J Physiol Regul Integr Comp Physiol* **280**: R771–R779.

Vasa M, Fichtscherer S, Adler K, Aicher A, Martin H, Zeiher AM *et al.* (2001). Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. *Circulation* **103**: 2885–2890.

Wagner AH, Ruckschloss U, Just I, Hecker M (2000). Improvement of nitric oxide-dependent vasodilataion by HMG-CoA reductase inhibitors through attenuation of endothelium superoxide anion formation. *Arterioscler Thromb Vasc Biol* **20**: 61–69.

Wassmann S, Laufs U, Bäumer AT, Müller K, Ahlbory K, Linz W *et al.* (2001). HMG-CoA reductase inhibitors improve endothelial dysfunction in normocholesterolemic hypertension via reduced production of reactive oxygen species. *Hypertension* **37**: 1450–1457.

Wassmann S, Laufs U, Müller K, Konkol C, Ahlbory K, Bäumer AT *et al.* (2002). Cellular antioxidant effects of atorvastatin *in vitro* and *in vivo*. *Arterioscler Thromb Vasc Biol* **22**: 300–305.

Werner ER, Werner-Felmayer G, Wachter H, Mayer B (1995). Biosynthesis of nitric oxide: dependence on pteridine metabolism. *Rev Physiol Biochem Pharmacol* **127**: 97–135.

Xu Z, Okamoto H, Akino M, Onozuka H, Matsui Y, Tsutsui H (2008). Pravastatin attenuates left ventricular remodelling and diastolic dysfunction in angiotensin II-induced hypertensive mice. *J Cadiovasc Pharmacol* **51**: 62–70.

Yamamoto A, Hoshi K, Ichihara K (1998). Fluvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, scavenges free radicals and inhibits lipid peroxidation in rat liver microsomes. *Eur J Pharmacol* **36**: 143–149.

Yang CM, Chien CS, Hsiao LD, Pan SL, Wang CC, Chiu CT *et al.* (2001). Mitogenic effect of oxidized low-density lipoprotein on vascular smooth muscle cells mediated by activation of Ras/Raf/MEK/MAPK pathway. *Br J Pharmacol* **132**: 1531–1541.

Ye P, Yu D, Song L, Deng X, Zhao Y (2000). Inhibitory effect of fluvastatin on aortic intimal thickening in normocholesterolemic rabbits. *Chin Med Sci J* **15**: 140–144.

Zhou R, Xu Q, Zheng P, Yan L, Zheng J, Dai G (2008). Cardioprotective effect of fluvastatin on isoproterenol-induced myocardial infarction in rat. *Eur J Pharmacol* **586**: 244–250.