Beneficial effect of simvastatin and pravastatin treatment on adverse cardiac remodelling and glomeruli loss in spontaneously hypertensive rats

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ABSTRACT
The aim of the present study was to investigate the possibility of different effects of the hydrophobic statin simvastatin and the hydrophilic statin pravastatin on the remodelling process in the overloaded left ventricle and renal cortex of SHRs (spontaneously hypertensive rats). Fifteen SHRs were treated for 40 days with simvastatin, pravastatin or placebo (water) via orogastric administration. Left ventricle and renal cortex were examined by light microscopy and stereology. LV (left ventricular) cardiomyocyte nuclei (N[cmn]) and glomeruli (N[gl]) numbers were estimated by the dissector method. BP (blood pressure) and serum triacylglycerols (triglycerides) were lower in the statin-treated groups than in the untreated control group. The volume density of the interstitial connective tissue was smaller and length density of the intramyocardial arteries, as well as the arteries/cardiomyocyte ratio, was greater in the statin-treated groups than in the control group. No difference was observed between the two statin-treated groups. The cross-sectional cardiomyocyte area was significantly smaller in the simvastatin-treated group than in the control or pravastatin-treated groups, and it was smaller in the pravastatin-treated group than in the control group. N[cmn] and N[gl] were greater in the two statin-treated groups than in the control group, but no significant difference was observed between the two statin-treated groups. In conclusion, administration of the statins simvastatin and pravastatin to SHRs effectively prevented the elevation in BP and serum triacylglycerols, and also attenuated adverse cardiac and kidney remodelling by preventing LV hypertrophy, enhancing myocardial vascularization with the decrease in interstitial fibrosis and attenuating cardiomyocyte and glomerular loss.

INTRODUCTION
The risk of fatal cardiovascular events is associated with an adverse structural remodelling of the myocardium, which is frequently caused by hypertensive heart disease [1]. Current hypertension management should not simply be focused only on a reduction in BP (blood pressure), but also on the adverse structural remodelling that generates hypertensive heart disease [2]. Cardiovascular diseases are also important risk factors that can cause renal function deterioration [3]. To minimize the cardiovascular risk associated with dyslipidaemia, the statins, or HMG-CoA key words: cardiac overload, statin, hypertension, nephrosclerosis, rat, remodelling, stereology.

Abbreviations: A[cm], cardiomyocyte mean cross-sectional area; ACE, angiotensin converting enzyme; Ang II, angiotensin II; BP, blood pressure; HDL, high-density lipoprotein; LV[art], length density of intramyocardial arteries; LV, left ventricular; LV/BM, LV mass/body mass ratio; MMP, matrix metalloproteinase; N[cmn], cardiomyocyte nuclei number; N[gl], glomerular number; NO, nitric oxide; SHR, spontaneously hypertensive rat.
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(3-hydroxy-3 methylglutaryl CoA) reductase inhibitors, are among the most frequently prescribed medication in clinical practice [4,5].

Recent studies support the notion that statins might improve LV (left ventricular) remodelling, preserve the ischaemic-reperfusion myocardium [6,7] and also prevent the development of hypertension and target organ damage independently of a cholesterol-lowering effect [8]. Experimental evidence has shown the amelioration of LV structural remodelling and contractile failure associated with the administration of statins are probably due to the inhibition of cardiac tissue ACE (angiotensin-converting enzyme) activity [9] and the attenuation of increased LV MMP (matrix metalloproteinase)-2 and -13 activity [10]. Treatment with statins was associated with a lower cardiac mass in patients with angina, suggesting that this is one of the pleiotropic effects of the drugs [11]. Other pleiotropic effects include the improvement in endothelial dysfunction, increased NO (nitric oxide) bioavailability, antioxidant effects, anti-inflammatory properties and stabilization of atherosclerotic plaques [12–14].

Some statins are hydrophobic (lovastatin, simvastatin, atorvastatin, fluvastatin and cerivastatin) and markedly reduce cell viability associated with DNA fragmentation, DNA laddering and activation of caspase-3, suggestive of apoptotic cell death. Pravastatin, which is a hydrophilic statin, however, did not induce apoptosis. Simvastatin also improved NO production and partially prevented the development of hypertension without preventing remodelling of the left ventricle and aorta in NO-deficient hypertension [15]. Pravastatin treatment reduced the incidence of cardiovascular events and coronary revascularization in patients with chronic renal insufficiency [16].

The aim of the present study was to investigate the possibility that the hydrophobic statin simvastatin and the hydrophilic statin pravastatin have different effects on the remodelling process in the overloaded left ventricle and renal cortex of SHRs (spontaneously hypertensive rats).

MATERIALS AND METHODS

Samples and procedures
Mature male SHRs (n = 15) were obtained from colonies maintained at the State University of Rio de Janeiro, individually housed in a temperature (21 ± 1 °C) and humidity–(60 ± 10 %) controlled room, submitted to a 12 h-dark/light cycle (artificial light, 07:00 to 19:00 hours) and air-exhaustion cycle (15 min/h). All procedures were carried out in accordance with the Conventional Guidelines for Experimentation with Animals (NIH Publication No. 85-23, revised 1996). The experimental protocols used in this study were approved by the Ethics Committee for Animal Experimentation, State University of Rio de Janeiro.

The animals were divided in three groups (n = 5 in each) and kept separately for 40 days as follows: (i) control group receiving food daily (Labina; Purina, Paraná, Brazil) and fresh water ad libitum; (ii) simvastatin group receiving 50 mg · day⁻¹ · kg⁻¹ of body weight simvastatin (Hexal, São Paulo, Brazil) via an orogastric tube; and (iii) pravastatin group receiving 50 mg · day⁻¹ · kg⁻¹ of body weight sodium pravastatin (Bristol-Myers Squibb, São Paulo, Brazil) via an orogastric tube.

The dose of statins used in the present study was based on a previous study [8] derived from experiments in which the production of H₂O₂ by polymorphonuclear leucocytes and aorta and plasma concentrations of creatine phosphate kinase were measured in Ang II (angiotensin II)-infused rats receiving statins at doses ranging from 1–120 mg/kg of body weight. Five animals per group characterized the minimum sample in quantitative studies as, if a parameter was found to increase (or decrease) in all five cases, then the probability that this was due to chance is P = (1/2)^5 < 0.05 and the experiment would be conclusive [17].

BP was recorded every week in conscious animals by tail-cuff plethysmography (Letica LE 5100; Panlab, Barcelona, Spain). On the morning of day 41 of experimentation, animals were anaesthetized (15 mg/kg of body weight sodium pentobarbital, intraperitoneally) and blood samples were taken by right atrium puncture. Serum triacylglycerols (triglycerides), HDL (high-density lipoprotein)-cholesterol, total cholesterol, creatinine, sodium and potassium levels were determined by the alkaline picrate method (Labset kit) using a Mega Bayer automatic analyser. The right atrium was then opened and a catheter put into the left ventricle to perfuse the vascular system with freshly prepared 4 % (w/v) formaldehyde in 0.1 M sodium phosphate buffer (pH 7.2) [18] at a pressure of 90 mmHg (Minipuls 3; Gilson, Villiers le Bel, France).

Tissue processing
After perfusion, the heart and left kidney were removed. The ventricles were dissected by separating the atria from the ventricles and the right ventricle from the left ventricle. The volumes of the left kidney, heart and left ventricle (including the interventricular septum) were determined according to the Sacherle’s submersion method [19] in which the water displacement due to organ volume was recorded by weighing. As the specific gravity (σ) of isotonic saline is 1.0048, the volume is obtained by the equation: volume = weight/σ, or simply volume = weight [20]. The LV/BM (LV mass/body mass) ratio was determined. Fragments of the left ventricle and left kidney were embedded in Paraplast plus® (Sigma, St. Louis, U.S.A.), sectioned at 3 (heart) or 5 (kidney) µm thickness and stained with haematoxylin/eosin (heart and kidney), Sirius Red (heart) or Masson Trichrome (heart and kidney).
Myocardial stereology
Isotropic myocardial sections were obtained by random cutting of the left ventricle [21]. The observer did not know the identification of the groups (sections were blinded by a technician). The myocardium was analysed considering cardiomyocytes and cardiac interstitium (composed of connective tissue with nerves and vessels). Volume densities of cardiomyocytes (Vv[cm]), connective tissue (Vv[ct]) and intramyocardial arteries (Vv[art]) were estimated by point counting:

\[ Vv(\text{structure}) = P_p(\text{structure}) / P_T \]

where \( P_p \) is the number of points hitting the structure (cardiomyocyte, connective tissue or intramyocardial arteries), and \( P_T \) is the total number of test-points (a 8600 \( \mu \)m\(^2\) frame with 36 test points was used). The cardiomyocyte and intramyocardial artery densities/area were estimated as the ratio between the number (n) of cardiomyocytes (cm) or intramyocardial arteries (art) and the test area (A\(_T\)) using the forbidden-line-exclusion protocol [22]:

\[ Q_v(\text{cm}) \text{ or [art]} = n(\text{structure}) / A_T \]

The length density of the intramyocardial arteries (Lv[art]) was estimated as:

\[ L_v(\text{art}) = 2 \times Q_v(\text{art}) \]

The cardiomyocyte mean cross-sectional area (A[cm]) was estimated from the equation:

\[ A_{\text{cm}} = Vv(\text{cm}) / 2 \times Q_v(\text{cm}) \]

and the intramyocardial artery/cardiomyocyte ratio was calculated as:

\[ Vv(\text{art}) / Vv(\text{cm}) \]

The numerical density of the cardiomyocyte nuclei (Nv[cmn]) was estimated with the optical dissector constructed with 20 \( \mu \)m apart (\( t \)), counting all glomeruli seen only in the ‘look-up plane’ (\( Q' \)) into a known frame (A\(_T\)) which did not intersect the forbidden line and the equation:

\[ N_v(\text{structure}) = Q' / t \times A_T \]

where \( P \) is the number of points hitting the structure (cardiomyocyte, connective tissue or intramyocardial arteries), and \( T \) is the total number of test-points. The product of Nv[g] and the renal cortical volume allowed the estimation of total glomerular number (Nv[g]).

Kidney stereology
The renal cortex was analysed according to the vertical section design [25]. The cortex/medulla ratio was estimated by point counting, and the Cavalieri method [23] was used to determine the volume of the renal cortex. The glomeruli numerical density (Nv[gl]) was estimated using the physical dissector constructed with 20 \( \mu \)m apart (\( t \)), counting all glomeruli seen only in the ‘look-up plane’ (\( Q' \)) into a known frame (A\(_T\)) which did not intersect the forbidden line and the equation:

\[ N_v(\text{structure}) = Q' / t \times A_T \]

Statistical analysis
The differences in the biometrical data were tested with the one-way ANOVA and the Newman–Keuls post-hoc test. The differences in the stereological data were tested by the non-parametric Kruskal–Wallis ANOVA and the Mann–Whitney test, because these data are from non-normal discrete variables (Bartlet’s test; GraphPad Prism version 4.02, San Diego, CA, U.S.A.). The significance level of 0.05 was used for statistical significance [26].

RESULTS
Treatment with high doses of statins was well tolerated by SHRs and no significant side effects were observed. The body mass of the SHRs during the experiment changed in each group (control group, from 340 (31) to 369 (27) g; simvastatin-treated group, from 345 (18) to 359 (27) g; and pravastatin-treated group, from 343 (19) to 365 (34) g; values are means (S.D.).)

In the untreated SHRs, hypertrophy of the myocardium and areas of ischaemia characterized by reactionary fibrosis with extensions around the intramyocardial arteries was observed (Figure 1A). These alterations were not seen in the myocardium of the statin-treated SHRs (Figures 1C and 1E). The renal cortex of untreated SHRs showed areas of fibrosis with inflammatory infiltrates, dilated blood vessels and glomeruli with increased cellularity (Figure 1B). The renal cortex of the statin-treated SHRs was preserved and showed a quite normal structure (Figures 1D and 1F).

BP
The BP, which was not different among the groups at the beginning of the study, was significantly lower (\(-11\%\); \( P < 0.05 \)) in the statin-treated groups compared with untreated control group at the end of the study. No difference was observed between the two statin-treated groups (Figure 2).

Quantitative study
The LV/BM ratio was decreased significantly in both the simvastatin (\(-20\%\)) and pravastatin (\(-14\%\))-treated groups when compared with the control group. Vv[ct] was smaller (\(-40\%\)) and the intramyocardial artery/cardiomyocyte ratio was significantly higher (+100\%; \( P < 0.05 \)) in the statin-treated groups [simvastatin-treated group, 18 (6)%; pravastatin-treated group, 18 (5)%] compared with the control group [9 (3)%]. There was no significant difference in Vv[cm] between any of the groups. Lv[art] was significantly greater in both the simvastatin (+100\%) and pravastatin (+70\%)-treated groups compared with the control group. No significant difference was observed between the two statin-treated groups. A[cm] was significantly lower (\( P < 0.05 \)) in the simvastatin-treated group compared with the control (\(-35\%\)) or pravastatin-treated (\(-25\%)\) groups (Table 1). A[cm] was significantly lower (\( P < 0.05 \)) in the pravastatin-treated group than in the control group (\(-20\%\); Table 1). Nv[cmn] was significantly higher (by approx. +30\%; \( P < 0.05 \)) in the statin-treated groups [simvastatin-treated group, 37.3 \( \times \) 10\(^6\) (3.4 \( \times \) 10\(^6\)); pravastatin-treated
In untreated SHRs, the myocardium (A) and the renal cortex (B) show areas of ischaemia with reactive fibrosis. The myocardium shows areas of ischaemic hypertrophy and increased interstitial fibrosis (arrow in A). The renal cortex shows areas of dilated vessels surrounded by increased interstitium with signs of inflammation (arrow in B) and glomeruli with increased cellularity. In statin-treated SHRs, the myocardium (simvastatin (C), and pravastatin (E)) shows small cardiomyocytes and few areas of interstitial fibrosis with preserved intramyocardial microvascularization (arrows in C and E). The renal cortex structure (simvastatin (D), and pravastatin (F)) is normal. (A–C) and (E) have the same magnification [bar in (E), 45 µm]. (D) and (F) have the same magnification [bar in (F), 90 µm].

In the left kidney, N[gl] was significantly greater (*P < 0.05) in the statin-treated groups [simvastatin-treated group, 31,563 (130); 477 (41)% greater; pravastatin-treated group, 604 (32)] compared with the control group [23,886 (230)]. No significant difference was observed between the simvastatin- and pravastatin-treated groups.

**DISCUSSION**

In the SHR strain used in the present study, BP elevation starts around weeks 12–14, and altered cardiac and renal

**Figure 2** BP evolution during the experiment
Values are means ± S.D. Both statin-treated groups were significantly different (*P < 0.05) than the control group after week 2.

**Table 1** Quantitative study of the myocardium
Values are means (S.D.). Differences were analysed using the Mann–Whitney test. *P < 0.05 when compared with the control group; †P < 0.05 when compared with the simvastatin-treated group; ‡P < 0.05 when compared with the pravastatin-treated group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Simvastatin-treated</th>
<th>Pravastatin-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vv[cm] (%)</td>
<td>82 (3)</td>
<td>81 (3)</td>
<td>81 (3)</td>
</tr>
<tr>
<td>Vv[ct] (%)</td>
<td>10 (2)</td>
<td>5 (1)*</td>
<td>4 (2)*</td>
</tr>
<tr>
<td>Lv[art] (µm/mm³)</td>
<td>1080 (130)</td>
<td>1700 (191)*</td>
<td>1350 (83)*</td>
</tr>
<tr>
<td>A[cm] (µm²)</td>
<td>789 (62)</td>
<td>477 (41)*</td>
<td>604 (32)*</td>
</tr>
<tr>
<td>LV/BM (mg/g)</td>
<td>3.2 (0.3)</td>
<td>2.6 (0.1)*</td>
<td>2.8 (0.4)*</td>
</tr>
</tbody>
</table>

**Serum analyses**

Serum HDL-cholesterol, total cholesterol, creatinine, sodium and potassium were not significantly different among the groups (Table 2). However, serum triacylglycerols were more than 30% lower in the statin-treated groups than in the control group. No significant difference in triacylglycerol levels was observed between simvastatin- and pravastatin-treated groups.
cortex remodelling occurs progressively, producing increased interstitial fibrosis and a gradual loss of LV cardiomyocytes [27], and glomerular rarefaction with hypertension of remaining glomeruli [28]. Statin treatment in these animals is efficient in preventing the elevation of BP and the associated adverse cardio–renal remodelling, irrespective of serum cholesterol levels.

Studies in humans suggest that the effects of statins on BP are probably restricted to patients with concomitant hypercholesterolaemia and uncontrolled hypertension [29,30], whereas animals studies have demonstrated beneficial effects of statins on BP independent of correction of hyperlipaemia [8,31]. Statins have no hypocholesterolaemic activity in mice [32], but statin treatment decreased triacylglycerol levels in stroke-prone SHRs [33]. The present study found that a high dose of statins significantly reduced triacylglycerol levels in SHRs, but without effecting HDL-cholesterol levels.

High-dose statin therapy has been shown to have a significant role in improving ventricular function in patients by improving endothelial flow [34]. Long-term use of fluvastatin by hyperlipidaemic hypertensive patients is associated with a significant reduction in aortic stiffness without any effect on BP [35]. High-dose atorvastatin (80 mg daily) potentiated the decline in inflammation in patients with acute coronary syndromes. This supports the value of early statin therapy in these patients [36]. Among patients who have recently had acute coronary syndrome, an intensive lipid-lowering statin regimen (80 mg of atorvastatin daily) provides greater protection against death or major cardiovascular events than does a standard regimen (40 mg of pravastatin daily). These findings indicate that such patients benefit from early and continued lowering of LDL-cholesterol to levels substantially below current target levels [37–39].

An anti-angiogenic effect of statins [40,41] might be mediated by RhoA [42] and are apparently dose-related. Low doses of statins may activate endothelial Ras and promote the phosphorylation of the protein kinase Akt/PKB (protein kinase B) and NO synthase, leading to an angiogenic effect, whereas higher statin doses are anti-angiogenic although they promote an increase in NO synthase protein expression [43]. This suggestion remains controversial because high doses of statins have also been shown to be angiogenic and further studies are necessary to clarify this [44].

In the development of LV hypertrophy, A(cm) may double, with a resultant change in the cell shape [45], and is influenced by haemodynamic (increased wall stress) and non-haemodynamic (altered genotypes) factors [46]. In the present study, LV hypertrophy was prevented/attenuated in statin-treated SHRs, as demonstrated by a smaller A(cm) and LV/BM ratio in comparison with untreated SHRs. Simvastatin apparently was more efficient than pravastatin in this regard. As both simvastatin- and pravastatin-treated SHRs reached the same BP at the end of the experiment, this action on A(cm) apparently did not depend on the BP. This agrees with the hypothesis that reduction in BP is not the only factor involved in the remodelling effects of statins considering that lovastatin, but not hydralazine (vasodilator), reduces renal vascular hypertrophy [31].

Structural remodelling of the myocardium in hypertension is collagenous in nature [47,48]. In the present study, treatment of SHRs with statins showed a positive effect on the cardiac interstitium as indicated by the small Vv[ct] seen in these two groups. Ang II is a mediator of the collagen matrix growth that can induce fibroblast proliferation and the increase in collagen synthesis by fibroblasts [49]. Statins probably prevent BP elevation by inhibiting Ang II production [8] and may attenuate the inflammatory effects of the risk of cardiovascular events [50]. Simvastatin reduced proliferation of cultured human atrial myofibroblasts independently of cholesterol synthesis via a mechanism involving inhibition of RhoA geranylgeranylation. Statins may therefore have an important role in preventing adverse myocardial remodelling associated with cardiac fibroblast proliferation [51].

In the present study, statins showed a beneficial effect on the myocardial vascularization that agrees with the idea that statin treatment improves myocardium perfusion [52]. Statins have been demonstrated to increase the number and migratory capacity of endothelial progenitor cells [53] and endothelial cell apoptosis (hydrophobic statins such as simvastatin) [54]. These effects of statins may play a role in angiogenesis [55] probably by enhancing NO synthesis [15,56]. The progressive loss of cardiomyocytes that usually occurs in SHRs until heart failure [57,58] was greatly attenuated in the present study, as observed by the main beneficial consequence of the treatment with statins on myocardial vascularization.

The association between fewer nephrons and hypertension is currently accepted [59]. Patients with hypertension have significantly fewer glomeruli per kidney.

### Table 2  Serum biochemistry

Values are means (S.D.). Differences were analysed using a one-way ANOVA and Newman–Keuls post-hoc test. *P < 0.05 when compared with the control group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>Simvastatin-treated</th>
<th>Pravastatin-treated</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>0.63 (0.16)</td>
<td>0.54 (0.06)</td>
<td>0.58 (0.08)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.60 (0.15)</td>
<td>0.53 (0.05)</td>
<td>0.55 (0.05)</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>0.30 (0.07)</td>
<td>0.21 (0.02)</td>
<td>0.19 (0.03)</td>
</tr>
<tr>
<td>Creatinine (µmol/l)</td>
<td>53 (12)</td>
<td>42 (4)</td>
<td>51 (4)</td>
</tr>
<tr>
<td>Na+ (mmol/l)</td>
<td>144 (5)</td>
<td>144 (7)</td>
<td>141 (1)</td>
</tr>
<tr>
<td>K+ (mmol/l)</td>
<td>5.8 (0.4)</td>
<td>5.8 (0.6)</td>
<td>5.3 (0.7)</td>
</tr>
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Prepared by: B. R. Bishop

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and greater glomerular volume than matched normotensive controls. The present study demonstrates that statin-treated SHRs have more glomeruli than untreated SHRs, which is associated with low BP levels at the end of the experiment. Although the renal abnormality underlying the development of hypertension in SHRs is apparently not due to inborn deficits in nephron endowment and/or filtration surface area [60,61], the finding that statin preservation of the number of glomeruli is associated with decreased BP is a positive effect of statin treatment in SHRs.

In conclusion, administration of the statins simvastatin and pravastatin to SHRs effectively prevented the elevation in BP and serum triacylglycerols, and also attenuated adverse cardiac and kidney remodelling by preventing LV hypertrophy, enhancing myocardial vasculization with decrease in interstitial fibrosis and attenuating cardiomyocyte and glomerular loss.

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REFERENCES


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40 Vincent, L., Chen, W., Hong, L. et al. (2001) Inhibition of endothelial cell migration by cerivastatin, an HMG-CoA reductase inhibitor: contribution to its anti-angiogenic effect. FEBS Lett. 495, 159–166

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