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Atorvastatin enhances sildenafil-induced vasodilation through nitric oxide-mediated mechanisms

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Abstract

Statins have cholesterol-independent effects including an increased vascular nitric oxide (NO) activity and are commonly used by patients with cardiovascular disease. Such patients frequently have erectile dysfunction, which may be treated with sildenafil, a selective inhibitor of phosphodiesterase type 5. Since statins and sildenafil can activate the NO-cGMP pathway, we investigated whether pre-treatment with atorvastatin (0, 5 and 30 mg/kg/day) for 2 weeks affects sildenafil (1 pM–100 mM)-induced relaxation of aortic rings isolated from Wistar rats. We also examined the hemodynamic consequences of this interaction in Wistar rats. Plasma nitrite/nitrate (NO_x) concentrations were determined using an ozone-based chemiluminescence assay. While pre-treatment with atorvastatin increased the potency of sildenafil-induced vasorelaxation ($P<0.01$), no differences were observed in the maximum sildenafil-induced relaxation. Pre-incubation of aortic rings with N^G-nitro-L-arginine methyl ester (L-NAME) reversed atorvastatin-induced increase in the potency of sildenafil relaxation. In addition, pre-treatment with atorvastatin enhanced plasma NO_x concentrations and sildenafil-induced hypotension and tachycardia (all $P<0.05$). These results suggest that atorvastatin increases the vascular sensitivity to sildenafil through NO-mediated mechanisms.

Keywords: Atorvastatin; Nitric oxide; Phosphodiesterase-5 inhibitor; Sildenafil; Statins; Vascular reactivity

1. Introduction

A class of drugs known as "statins" shares a common mechanism of action that involves the inhibition of cholesterol synthesis in the liver by blocking the conversion of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) to mevalonate, the rate-limiting step in the cholesterol synthesis (Goldstein and Brown, 1990). However, in addition to their cholesterol lowering properties, statins have been proposed to have additional, cholesterol-independent, beneficial effects that are encountered early in the course of lipid lowering therapy (Bonetti et al., 2003). These so-called pleiotropic effects of statins include an increased expression of endothelial nitric oxide synthase (eNOS) that is mediated through increases in eNOS mRNA stability (Laufs et al., 2000; Laufs et al., 1998; Laufs et al., 2001). As a consequence of the increase in eNOS expression, statins enhance nitric oxide (NO) production by endothelial cells (Lefer et al., 2001). Moreover, statins can increase NO bioavailability through activation of the serine/threonine kinase Akt which, in turn, phosphorylates eNOS (Kureishi et al., 2000) and through a decrease in caveolin abundance (Feron et al., 2001). These observations provide strong evidence that statins can increase vascular NO activity and significantly improve endothelial vasodilatory functions (Treasure et al., 1995). Indeed, previous studies have

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demonstrated direct vasorelaxing effects of statins through inhibition of Ca^{+2} mobilization and activation of K^{+} channels in vascular smooth muscle cells, and by increasing endothelium-derived NO (Alvarez De Sotomayor et al., 2000; Alvarez De Sotomayor et al., 2001; Mochida et al., 2002; Mukai et al., 2003).

Many clinical trials have demonstrated that statins can prevent cardiovascular events in patients at increased cardiovascular risk. Such patients frequently have erectile dysfunction, which is commonly treated with sildenafil, a selective inhibitor of cGMP-specific phosphodiesterase type 5 (PDE5) that causes modest reductions in arterial blood pressure (Gillies et al., 2002) through increased cGMP levels. Although the cardiovascular effects and many of the drug–drug interactions of sildenafil have already been addressed (Cheitlin et al., 1999), no previous study has focused on the possible interaction of sildenafil with statins. Such an interaction could result from the fact that both drugs activate the NO-cGMP pathway, thereby producing vasodilation (Webb et al., 2000). In the present study, we investigated whether pre-treatment with atorvastatin for 2 weeks affects the vascular reactivity to sildenafil. We also examined the hemodynamic consequences of this interaction.

2. Materials and methods

2.1. Animals and pre-treatment with atorvastatin

The study complied with international guidelines of the European Community for the use of experimental animal and was approved by the institutional ethics committee. Male Wistar rats (190–210 g) obtained from the colony at University of Sao Paulo (Campus of Ribeirao Preto) were maintained on a 12-h light/dark cycle at a room temperature (22–25 °C) with free access to standard rat chow and water. The animals were randomly assigned to one of three experimental groups as follows: rats that received tap water (controls), and two groups of rats that received atorvastatin (5 or 30 mg/kg/day; p.o.) for 2 weeks.

2.2. Study 1: Effect of atorvastatin on the vascular responses to sildenafil

2.2.1. Preparation of rat aortic rings

The rats were killed by decapitation and the thoracic aorta was isolated and cleaned of connective tissue and fats. Aortic rings, 4 mm in length, were cut and mounted for isometric tension recording. The rings were placed in bath chambers (10 ml) for isolated organs containing modified Krebs salt solution (KSS) of the following composition (mM): NaCl 130, $CaCl_2$ 1.6, $MgSO_4$ 1.2, KH_2PO_4 1.2, KCl 4.7, $NaHCO_3$ 14.9, glucose 5.5, which was maintained at 37 °C, pH 7.4, and bubbled with 95% O_2 and 5% CO_2. The system was connected to a Letica force displacement transducer and the responses were recorded on a computer system using the Chart V4.04. PowerLab ADInstruments (2000) Program. The aortic rings were submitted to a tension of 1.5 g during the 60-min equilibration period and were considered to have an intact functional endothelium when acetylcholine (1 μM) produced a relaxation of more than 80%. Relaxation was calculated as a percentage of the contraction induced by phenylephrine (1 μM).

2.2.2. Concentration–response curves to sildenafil

After the equilibration period, the aortic rings were contracted with 1 μM phenylephrine and the contraction was allowed to stabilize. After washout, some rings were incubated with N^{G}-nitro-L-arginine methyl ester (L-NAME; 10 μM) for 30 min while control arterial rings received no treatment. The rings were then contracted again with 1 μM phenylephrine and cumulative concentration–response curves for sildenafil (1 pM–100 mM) were constructed. This procedure was performed in aortic rings obtained from rats that were pre-treated with tap water (controls), or atorvastatin (5 or 30 mg/kg/day; p.o.) for 2 weeks.

2.3. Study 2: Effect of atorvastatin on the hypotensive responses to sildenafil

2.3.1. General procedures

The rats were anesthetized with urethane (1 g/kg, i.p.) and the trachea was cannulated with a Gelco tube. The right carotid artery and left femoral vein were cannulated for the measurement of arterial blood pressure and drug administration, respectively. The arterial catheter was connected to a COBE transducer (Arvada, CO), and the signal was amplified with a GP4A-general purpose amplifier (Stemtech, MacLab/PowerLab, Milford, MA). The amplifier outputs were connected to an A/D board and this to a computer loaded with CODAS data acquisition software (AT-CODAS; DATAQ Instruments, Akron, OH). The pulsatile arterial pressure was recorded continuously at a sample rate of 200 Hz throughout the experiment. The experiments were initiated after at least 20 min of stabilization.

2.3.2. Sildenafil administration

Each dose of sildenafil was dissolved in saline and was given in 100 μl intravenous (i.v.) bolus, then washed in with a further 100 μl of saline. All rats received saline followed by sildenafil at doses of 0.1, 0.3, 1, and 3 mg/kg, each dose given every 5–7 min. The doses of sildenafil we selected in the present study were based on the previous studies demonstrating that similar doses (from 0.1 to 1 mg/kg) produced dose-dependent decreases in mean arterial pressure and increases in heart rate (Schwemmer et al., 2001). However, such dose–response behaviour was not found in other studies (Gillies et al., 2002; Sugiyama et al., 2001).

The changes in mean arterial pressure were calculated as the differences between the baseline values and those

recorded at the lowest values of mean arterial pressure after each dose of sildenafil. Similarly, the changes in heart rate were calculated as the differences between the baseline values and those recorded at the highest values of heart rate after each dose of sildenafil.

2.4. Measurement of plasma nitrite/nitrate concentrations

Arterial blood samples were collected in tubes containing EDTA. After blood centrifugation at 800×*g* for 5 min, plasma aliquots were removed and stored at −20 °C until analyzed in duplicates for their nitrite and nitrate (NO_x) content using an ozone-based chemiluminescence assay (Reiter et al., 2002). Briefly, the plasma samples were treated with a 2:1 volume of cold ethanol and centrifuged at 14,000×*g* for 5 min. NO_x were measured by injecting 25 μl of the supernatant in a glass purge vessel containing vanadium (III) in 1 N hydrochloric acid at 90°, which reduces NO_x to NO gas. A nitrogen stream was bubbled through the purge vessel containing vanadium (III), then through 1 N NaOH, and then into a NO analyzer (Sievers Model 280 NO Analyzer, Boulder, CO, USA), which detects NO released from NO_x for chemiluminescent detection.

2.5. Drugs

Urethane, acetylcholine, phenylephrine, L-NAME, and vanadium (III) were purchased from Sigma (St. Louis, MO, USA). Sildenafil and atorvastatin were provided by Pfizer (São Paulo, SP, Brazil).

2.6. Statistical analysis

All the results are expressed as means±S.E.M. One-way analysis of variance followed by the Student–Newman–Keuls test was used to analyze differences in vascular reactivity and in plasma NO_x concentrations among groups (SigmaStat for Windows, Jadel Scientific, USA). The between groups comparisons of hemodynamic data were assessed by two-way analysis of variance (ANOVA). A probability value <0.05 was considered the minimum level of statistical significance.

3. Results

3.1. Study 1: Effect of atorvastatin on the vascular responses to sildenafil

Sildenafil (1 pmol/l–100 mmol/l) induced relaxation in a concentration-dependent way in rat aortic rings precontracted with phenylephrine (Fig. 1). Interestingly, treatment with atorvastatin (30 mg/kg/day) for 2 weeks increased the potency of sildenafil compared to control rats (pD2 increased from 6.61±0.47 to 8.28±0.04; $P<0.01$; $n=5$ in both groups; Figs. 1 and 2A). Likewise, treatment with a lower dose of atorvastatin (5 mg/kg/day) produced a similar increase in the potency of sildenafil-induced relaxation to that produced by 30 mg/kg/day (pD2 increased from 6.61±0.47 to 7.77±0.08; $P<0.05$; $n=5$ in both groups; Fig. 2A).

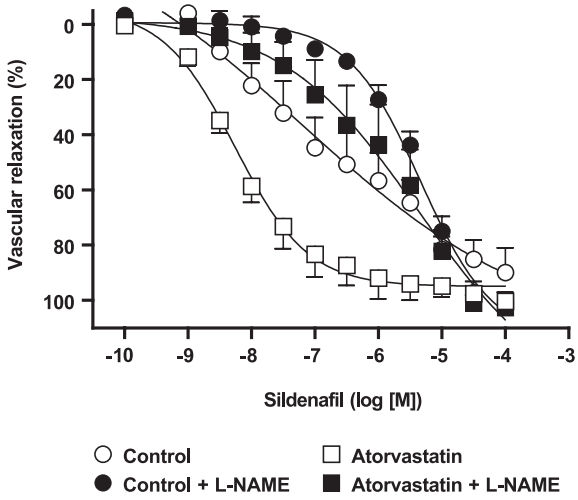

Fig. 1. Vascular relaxation induced by sildenafil in rat aortic rings. Concentration–effect curves for sildenafil (1 pmol/l–100 mmol/l) were constructed in phenylephrine-contracted aortic rings obtained from control and atorvastatin (30 mg/kg/day)-treated rats in the absence or after incubation with L-NAME (10 μmol/l) for 30 min. Treatment with atorvastatin enhanced the potency of sildenafil-induced vascular relaxation in rat aortic rings. Incubation with L-NAME reversed atorvastatin-induced effect.

Incubation of aortic rings with L-NAME reduced the potency of sildenafil-induced relaxation in the three experimental groups. As shown in Fig. 2A, pD2 decreased from 6.61±0.47 to 5.36±0.04 in control aortic rings, and from 7.77±0.08 to 6.42±0.18 in aortic rings from rats treated with atorvastatin 5 mg/kg/day ($n=5$ or 6/group; $P<0.05$ and $P<0.01$, respectively). A more pronounced decrease in pD2 after incubation with L-NAME was observed in aortic rings from rats pre-treated with atorvastatin 30 mg/kg/day (from 8.28±0.04 to 5.72±0.38; $n=5$ in both groups; $P<0.01$; Figs. 1 and 2A).

While significant differences were observed in the potency of sildenafil-induced relaxation of aortic rings from rats treated with both doses of atorvastatin, no differences were observed in the maximum relaxation obtained with sildenafil in the three experimental groups (Fig. 2B). In addition, incubation of aortic rings with L-NAME did not affect the maximum relaxation obtained with sildenafil in the three experimental groups (Fig. 2B).

3.2. Study 2: Effect of atorvastatin on the hypotensive responses to sildenafil

Table 1 shows that treatment with atorvastatin 5 or 30 mg/kg/day did not affect body weight, baseline mean arterial pressure and heart rate.

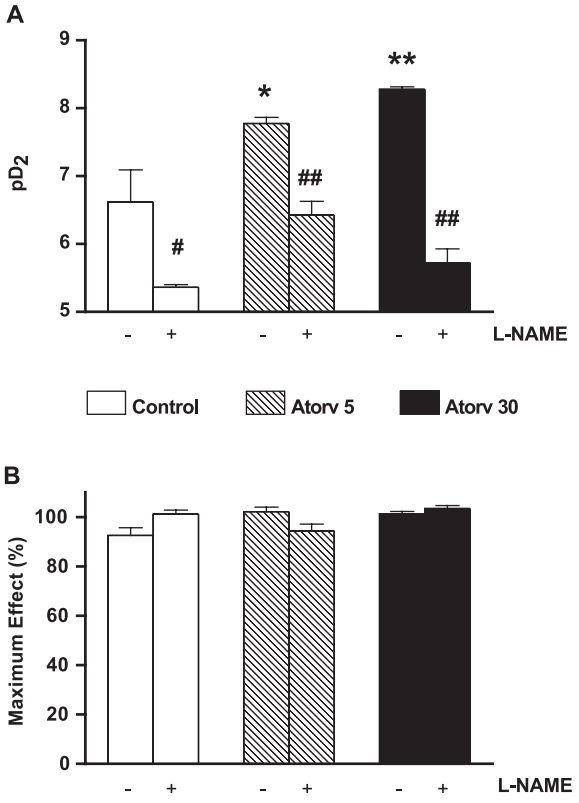

Fig. 2. Values of pD_2 (panel A) and maximum effect (panel B) of sildenafil in rat aortic rings. The values were obtained in aortic rings obtained from control and atorvastatin (5 or 30 mg/kg/day)-treated rats in the absence (−) or after incubation (+) with L-NAME (10 μmol/l) for 30 min (n=5 or 6 per group). *P<0.05 versus the control group. **P<0.01 versus the control group. $^{\#}P$<0.05 versus in the absence (−) of L-NAME. $^{\#\#}P$<0.01 versus in the absence (−) of L-NAME.

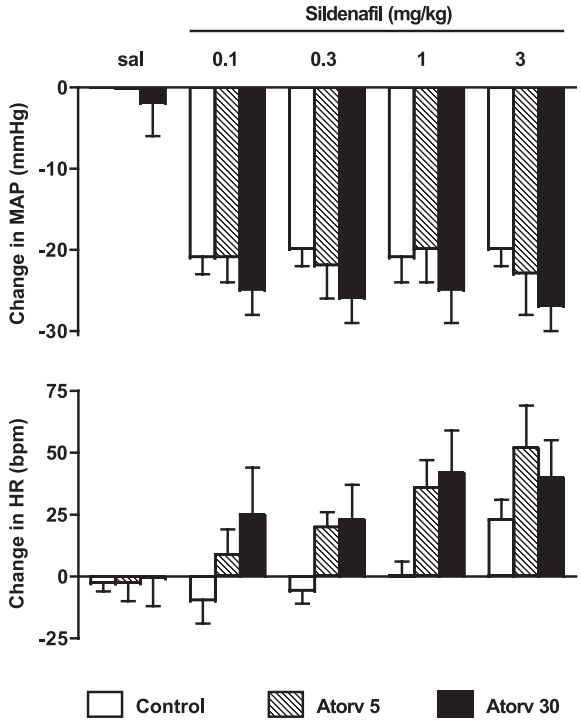

Fig. 3. Change in mean arterial pressure (MAP) and heart rate (HR) caused by the i.v. injection of saline (sal) or sildenafil (0.1, 0.3, 1, 3 mg/kg) in anesthetized control and atorvastatin (5 and 30 mg/kg/day; Atorv 5 and Atorv 30, respectively)-treated rats. Values are the mean±S.E.M.

The two-way ANOVA showed no significant pre-treatment×sildenafil dose interaction for the MAP and HR (F(8,100)=0.14 and F(8,100)=0.75, respectively; both P>0.05; n=7–10 rats per group). However, sildenafil significantly reduced mean arterial pressure and increased heart rate in the three groups of rats (both P<0.01; Fig. 3A and B). While only pre-treatment with atorvastatin 30 mg/kg/day enhanced sildenafil-induced hypotensive effects (P<0.05), both doses of atorvastatin enhanced sildenafil-induced tachycardic responses (both P<0.05).

Table 1
Body weight (BW), baseline mean arterial pressure (MAP) and heart rate (HR) in anesthetized rats treated with atorvastatin 5 (Atorv 5) or 30 (Atorv 30) mg/kg/day

	Control	Atorv 5	Atorv 30
BW (g)	290±11	296±14	301±15
MAP (mm Hg)	94±3	88±4	91±3
HR (bpm)	400±16	384±15	392±23
N	10	6	7

Control rats received tap water.
Values are the mean±S.E.M.

3.3. Effects of pre-treatment with atorvastatin on plasma nitrite/nitrate concentrations

Pre-treatment with atorvastatin 5 or 30 mg/kg/day significantly increased plasma nitrite/nitrate concentrations (both P<0.05; Fig. 4).

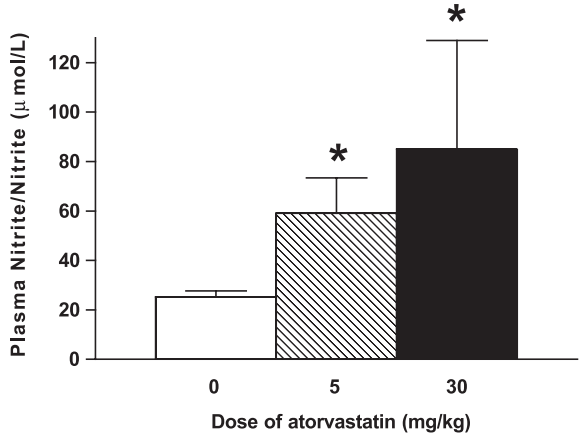

Fig. 4. Concentrations of nitrite/nitrate in plasma from control and atorvastatin (5 and 30 mg/kg/day; Atorv 5 and Atorv 30, respectively)-treated rats. Values are the mean±S.E.M. *P<0.05 versus the control group.

4. Discussion

The major findings of this study were that (i) pre-treatment with atorvastatin (5 or 30 mg/kg/day for 2 weeks) increased the potency of sildenafil-induced vasorelaxation; (ii) inhibition of NO synthesis with L-NAME reversed the increase in vasorelaxation induced by atorvastatin; (iii) pre-treatment with atorvastatin 30 mg/kg/day enhanced sildenafil-induced hypotensive effects.

There has been much concern about possible interactions of sildenafil with other drugs (Sakuma et al., 2002), especially in patients at increased cardiovascular risk. This is because sildenafil-induced increases in cGMP levels can potentiate cGMP-mediated dilator responses to other drugs that activate the NO-cGMP pathway and cause severe systemic hypotension and death. For example, the vasodilator actions of nitrates are potentiated with concomitant use of sildenafil (Cheitlin et al., 1999; Ishikura et al., 2000). Therefore, it is generally accepted that sildenafil should not be used by patients taking nitrates or other NO donors, regardless of their hemodynamic site of action, because of the risk of developing potentially life-threatening hypotension (Cheitlin et al., 1999). However, although statins are known to increase eNOS expression and to enhance vascular NO activity (Alvarez De Sotomayor et al., 2000; Mochida et al., 2002; Mukai et al., 2003; Feron et al., 2001; Kureishi et al., 2000), significantly improving endothelial vasodilatory functions (Treasure et al., 1995), no previous study has addressed the possible cardiovascular interactions of statins with sildenafil.

In this study, atorvastatin increased the potency of sildenafil-induced vasorelaxation. Our data show that the concentrations of sildenafil required to produce half of the maximum effect are more than 10 times lower in animals pre-treated with both doses of atorvastatin compared with control animals. However, the maximum vasorelaxation was not affected by the pre-treatment with atorvastatin. Taken together, these findings suggest that atorvastatin increased the sensitivity of vascular tissues to sildenafil, without significantly affecting the efficacy of this drug. This increase in vascular sensitivity to sildenafil is easily explained by an increased vascular NO activity in aortic rings from rats pre-treated with statins. (Alvarez De Sotomayor et al., 2000; Mochida et al., 2002; Mukai et al., 2003; Lefer et al., 2001). Giving further support to this hypothesis, the increased plasma nitrate/nitrite concentrations we found in rats pre-treated with both doses of atorvastatin may reflect an increased expression of eNOS and an enhanced NO production. Many other previous studies have also demonstrated that treatment with statins can increase NO production (Bonetti et al., 2003; Coelho-Filho et al., 2001; Kalinowski et al., 2002). In addition, incubation of aortic rings with L-NAME, a non-selective inhibitor of NO synthesis, reduced the potency of sildenafil-induced relaxation (Mochida et al., 2002; Sakuma et al., 2002), especially in aortic rings from rats pre-treated with the higher dose of atorvastatin, thus suggesting that atorvastatin-induced increase in vascular sensitivity to sildenafil is mediated through NO-dependent mechanisms.

Considering the significant increase in vascular sensitivity to sildenafil we found after a 2-week pre-treatment with atorvastatin (study 1), we decided to examine the hemodynamic consequences of this changes in vascular reactivity (study 2). Interestingly, pre-treatment with atorvastatin 30 mg/kg/day enhanced sildenafil-induced hypotensive effects. Although the small differences in sildenafil-induced hypotension among groups could be considered of little clinical significance, it should be taken into account that we studied normal (not hypertensive) rats. Curiously, the reflex tachycardia to the injections of sildenafil was more intense in animals pre-treated with atorvastatin. Consistent with an increased sildenafil-induced vasorelaxation in rats pre-treated with atorvastatin, the increased tachycardic responses in these animals may have attenuated the hypotensive responses to sildenafil (Sugiyama et al., 2001). This finding could explain why the differences in blood pressure lowering effects of sildenafil were small. Finally, previous studies have shown that sildenafil does not have any direct inotropic effect on dog or human heart (Corbin et al., 2003). Therefore, our findings presumably reflect the vascular interactions between atorvastatin and sildenafil.

In conclusion, our results suggest that atorvastatin can increase the bioavailability of NO and enhance the sensitivity of sildenafil-induced vasorelaxaton. Because the differences in sildenafil-induced hypotension were small, we believe that this effect is probably of minor clinical significance in terms of increasing cardiovascular events. A clinical study addressing this issue is warranted.

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