Rosuvastatin augments the beneficial hemodynamic effects of valsartan in nitric oxide-deficient hypertensive rats

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Objective
The possible beneficial effects of the association between rosuvastatin (3-hydroxy-3-methylglutaryl coenzyme reductase inhibitor) and valsartan [angiotensin receptor blocker (ARB)] on arterial blood pressure, endothelial nitric oxide production, cardiac hypertrophy, and lipid profile in nitric oxide-deficient hypertensive rats were examined.

Background
Statins and ARB possess common additional properties such as restoration of endothelial activity and antioxidant properties. These properties eventually prove useful for the improved treatment of cardiovascular disease.

Method
Hypertension was induced in male albino Wistar rats by daily gavage of NG-nitro-L-arginine-methyl ester (L-NAME, 50 mg/kg) for 3 weeks. These animals were randomly assigned to the following groups: L-NAME, L-NAME/valsartan, L-NAME/rosuvastatin, and L-NAME/valsartan + rosuvastatin.

Result
Oral administration of L-NAME for 3 weeks induced significant elevation in arterial blood pressure and increased the heart rate but did not show any significant change in plasma lipid profile. Meanwhile, plasma nitric oxide level was reduced to 20% of its normal level, and the plasma malondialdehyde level was significantly increased by 33.21%. Coadministration of rosuvastatin with valsartan improved hypertension, normalized the heart rate, increased plasma nitric oxide level by 70.06%, and restored the plasma malondialdehyde level to its normal value.

Conclusion
Coadministration of valsartan (ARBs) and rosuvastatin (3-hydroxy-3-methylglutaryl coenzyme reductase inhibitor) as primary treatment therefore provides a greater degree of protection, controls the risk factors, and improves the vascular and general health.

Keywords:
angiotensin receptor blockers, hypertension, statins

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Introduction

Hypertension is a complex pathophysiological state that manifests itself as chronic high blood pressure and is a major risk factor for many cardiovascular diseases (CVDs) such as stroke, heart failure, coronary artery disease, and progressive renovascular damage [1,2].

Hypertension frequently coexists with other cardiovascular risk factors such as hypercholesterolemia, and their combined effect is associated with a higher rate of cardiovascular events. Various clinical data support the fact that treatment of hypertensive (HT) patients with a combination of anti-HT and lipid-lowering therapies leads to a higher reduction in the incidence of cardiovascular events [3].

In hypertension, the delicate balance between vasodilators and vasoconstrictors is upset, with disturbance in the nitric oxide (NO) pathways that leads to a predominance of vasoconstrictors. This may lead to a vicious cycle that maintains high blood pressure and produces end-organ damage [4,5].

In rats, a sustained and reversible systemic hypertension can be induced by chronic inhibition of endothelial NO production using L-arginine analogs such as NG-nitro-L-arginine-methyl ester (L-NAME) [6]. Although the precise pathogenesis of L-NAME hypertension remains unknown, its development requires an intact renin–angiotensin system (RAS) [7,8]. RAS contributes markedly to a variety of CVD and is the target of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers (ARBs) [9]. They act by blocking the angiotensin-1 (AT1) receptors that mediate most of the cardiovascular effects of angiotensin II, including oxidative stress, vasoconstriction, and cardiac and vascular cell hypertrophy [10,11].

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The 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) exert both direct and indirect cholesterol-lowering effects on the vasculature. Statins have been shown to significantly reduce cardiovascular mortality and morbidity in patients at risk for CVD [12,13]. It has also been suggested that statins may have direct effects on plaque stability, NO metabolism, inflammation, endothelial function, oxidative stress, and stroke [13,14].

On the basis of the previous work, this study was carried out to investigate the possible impact of rosuvastatin (HMG-CoA reductase inhibitor) on the beneficial hemodynamic effect of valsartan (ARB) in NO-deficient HT rats.

Subjects and methods

Materials

Animals

Adult male albino Wistar rats weighing 200–280 g were used in the present study. They were purchased from the Animal House Colony of the National Research Center, Cairo, Egypt. Animals were housed under standardized conditions (room temperature 23 ± 2°C; relative humidity 55 ± 5%; 12 h light/dark cycle) and have free access to tap water and standard rat chow throughout the whole experimental period. All animal procedures were performed after the Ethics Committee of the National Research Center and in accordance with the recommendations for the proper care and use of laboratory animals (Canadian Council on Animal Care Guidelines, 1984).

Chemicals and drugs

L-NAME (Acros Organics, Ceel, Belgium), urethane (Sigma-Aldrich Chemie, Munich, Germany), thiobarbituric acid (TBA; Merck, Darmstadt, Germany), perchloric acid (Sigma Chemical company, Saint Louis, MO, USA), trichloroacetic acid (Fluka Chemie AG, Buchs, Switzerland), total NO kit (BioAssay Systems, Hayward, CA, USA), and cholesterol, triglyceride, and HDL-cholesterol kits (Biodiagnostic, Cairo, Egypt). Rosuvastatin (IPR, Pharmaceutical Inc., Peurto Rico; Astrazeneca, UK) and valsartan were obtained as gifts from Global Napi Pharmaceutical company (6 of October City, Egypt).

Experimental design and treatment protocol

After 7 days of adaptation to laboratory conditions, the animals were randomly assigned to experimental groups consisting of 8–10 rats each. Animals were randomly classified according to the following design:

Group 1: Normal rats that received distilled water and served as normotensive controls.

Chronic hypertension was induced in male albino Wistar rats by daily gavage of L-NAME at a daily dose of 50mg/kg/day for 3 weeks [15]. Treatment was carried out once in the morning before supplying food to the animals to allow best absorption of the agent.

In a randomly selected subset of eight animals, the time course of the increase in arterial blood pressure (ABP) was assessed along the 3 weeks of the treatment period: ABP was invasively measured at the end of the first, second, and third weeks. Under our experimental conditions, a sustained elevation of ABP was achieved in the third week. Animals with blood pressure over 160 mmHg were considered HT and selected for the study. HT rats were randomly assigned to the following groups:

Group 2: Group 2 was the HT control group.

Group 3: Group 3 comprised HT rats that received valsartan (10 mg/kg/day, orally) for 3 weeks [16].

Group 4: Group 4 comprised HT rats treated with rosuvastatin (10 mg/kg/day, orally) for 3 weeks [17].

Group 5: Group 5 comprised HT rats treated with valsartan (10 mg/kg/drug/day, orally) and rosuvastatin (10 mg/kg/drug/day, orally) for 3 weeks.

Treatment with valsartan, rosuvastatin, or their combination started at the beginning of the first week and continued together with L-NAME for 3 weeks.

Measurement of arterial blood pressure

At the end of the treatment period (3 weeks), the rats' body weights were recorded. They were then anesthetized with urethane (1.5 g/kg, intraperitoneally) [18,19], and a polyethylene catheter (1.0 mm outer diameter) attached to a pressure transducer (Isotec; Hugo Sachs Elektronik, March-Hugstetten, Germany) was implanted into the left carotid artery for recording of ABP and heart rate following the method described by Krzeminski *et al.* [20]. The transducer was connected to a pressure coupler (Type 566; Hugo Sachs Elektronik) mounted on an oscillographic recorder (Linear mark VI, Graphtec Corporation, March-Hugstetten, Germany). Mean arterial blood pressure (MABP) was calculated according to the following equation:

$$\mathrm{MABP} = \mathrm{DBP} + 1/3\ (\mathrm{SBP} - \mathrm{DBP}),$$

where DBP is the diastolic blood pressure and SBP the systolic blood pressure.

At the end of the measurement procedure, blood samples were collected directly from the carotid artery. Plasma samples were obtained by centrifugation at 3500 rpm at 8°C for 20 min (Hermle Labortechnik, type Z 323 K, Wehingen, Germany).

The animals were killed; the heart was isolated, plotted between two filter papers, weighed, and the heart weight/body weight ratio was calculated.

Biochemical estimations

Total cholesterol and triglyceride levels were estimated in plasma according to the method described by Allain *et al.* [21] and Fassati and Prencipe [22], respectively. HDL was assayed according to the method of Lopes-Virella *et al.* [23] and Gordon and Gordon [24]. The LDL cholesterol level was calculated according to the equation of Friedewald *et al.* [25]:

$$\mathrm{LDLcholesterol} = \mathrm{Total\ cholesterol} - (\mathrm{HDLcholesterol} + \mathrm{triglyceride}/5)$$

Table 1 Effect of 3 weeks of oral daily administration with valsartan, rosuvastatin, or their combination on SBP, DBP, MABP, and heart rate in L-NAME-induced hypertensive male rats

	Parameters Mean ± SEM			
Groups	SBP (mmHg)	DBP (mmHg)	MABP (mmHg)	Heart rate (beats/min)
Normal (distilled water)	106.67[a] ± 5.42	67.50[a,b] ± 7.04	80.55[a,b] ± 4.80	283.16[a] ± 18.43
HT-control (L-NAME, 50 mg/kg/day)	168.12*[b] ± 2.09	128.75*[b] ± 3.09	141.75*[b] ± 2.58	402.00*[b] ± 7.49
HT-valsartan (10 mg/kg/day)	143.75*[a,b] ± 4.97	120.62*[b] ± 4.85	128.25*[a,b] ± 4.63	348.00*[a] ± 15.23
HT-rosuvastatin (10 mg/kg/day)	133.12*[a,b] ± 3.65	105.00*[a,b] ± 5.26	114.25*[a,b] ± 4.57	330.00[a] ± 14.99
HT-valsartan + rosuvastatin (10 mg/kg/day/drug)	118.33[a] ± 3.07	90.00*[a] ± 3.65	99.39*[a] ± 2.53	290.00[a] ± 11.24

Each value represents the mean value ± SEM of the number of animals in each group (n = 6–8).
DBP, diastolic blood pressure; HT, hypertensive; L-NAME, NG-nitro-L-arginine-methyl ester; MABP, mean arterial blood pressure; SBP, systolic blood pressure.
*Significantly different from the corresponding normal value at P up to 0.05.
[a]Significantly different from hypertensive control value at P up to 0.05.
[b]Significantly different from valsartan + rosuvastatin-treated value at P up to 0.05.

Total NO metabolites (nitrate + nitrite) were assessed in plasma according to the method described by Bulau *et al.* [26] and Hasegawa *et al.* [27]. In this assay, cadmium quantitatively reduces nitrate to nitrite. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess reaction. The Griess reaction is based on the two-step diazotization reaction in which acidified NO_2^- produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to *N*-(1-naphthyl) sulfanilic acid to form the chromophoric azo derivative, which absorbs light at 540 nm.

Lipid peroxides were estimated colorimetrically by TBA reaction as described by Yagi [28]. TBA reacts with malondialdehyde (MDA) in acidic medium at 95°C for 30 min to form TBA reactive product. The absorbance of the resultant pink product can be measured at 534 nm.

Statistical analysis

Results were expressed as means ± SEM. Statistical analysis of the obtained data was performed using SPSS statistical software, release 16.00 (SPSS Inc., Chicago, IL, USA). The one-way analysis of variance test was carried out, followed by determination of post-hoc least significance difference. For all tests, statistical significance was set at P up to 0.05.

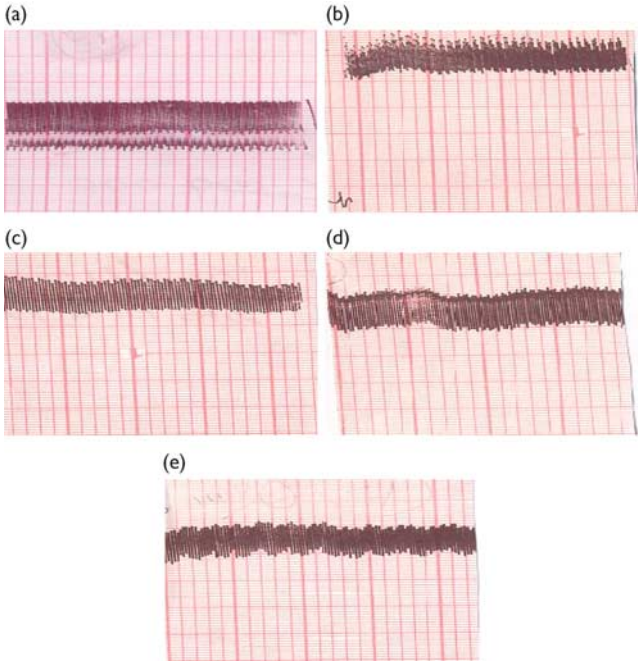
Results

Arterial blood pressure

The results present in Table 1 and illustrated in Fig. 1 reveal that daily oral administration of L-NAME (50 mg/kg) for 3 weeks induces a marked significant elevation in SBP, DBP, and MABP by 80.54, 116.82, and 101.06%, respectively, compared with the corresponding normal values. Daily supplementation of valsartan (10 mg/kg/day) for 3 weeks reduced SBP, DBP, and MABP by 14.49, 6.31, and 9.52%, respectively.

A similar effect was obtained in the rosuvastatin-treated group, in which SBP, DBP, and MABP levels were significantly reduced by 20.81, 18.44, and 19.40%, respectively. Coadministration of valsartan and rosuvastatin produced an additive reduction in SBP, DBP, and MABP to 118.33 ± 3.07, 90.00 ± 3.65, and 99.39 ± 2.53 mmHg, respectively, values that are statistically lower than those produced by valsartan alone.

Figure 1

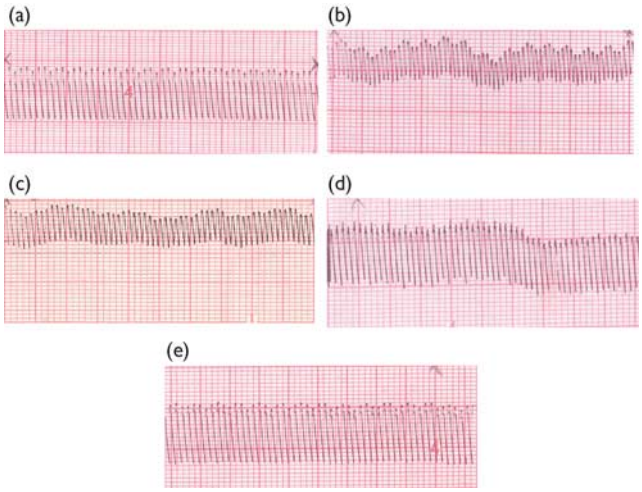


Examples of recordings of arterial blood pressure (mmHg) following 3 weeks of oral treatment with valsartan, rosuvastatin, or their combination in L-NAME-induced hypertensive male rats. (a) Normal (SBP = 106.67 ± 5.42 and DBP = 67.50 ± 7.04 mmHg); (b) L-NAME, 3 weeks (SBP = 168.12 ± 2.09 and DBP = 128.75 ± 3.09mmHg); (c) valsartan (SBP = 143.75 ± 4.97 and DBP = 120.62 ± 4.85 mmHg); (d) rosuvastatin (SBP = 133.12 ± 3.65 and DBP = 105.00 ± 5.26 mmHg); (e) valsartan + rosuvastatin (SBP = 118.33 ± 3.07 and DBP = 90.00 ± 3.65 mmHg). DBP, diastolic blood pressure; L-NAME, NG-nitro-L-arginine-methyl ester; SBP, systolic blood pressure.

Heart rate

Regarding the results of the heart rate, data present in Table 1 and illustrated in Fig. 2 show that the normal heart rate was 283.16 ± 18.43 beats/min. Daily gavage of

Figure 2



Examples of recordings of heart rate (beats/min) following 3 weeks of oral treatment with valsartan, rosuvastatin, or their combination in L-NAME-induced hypertensive male rats. (a) Normal (283.00 ± 18.43 beats/min); (b) L-NAME, 3 weeks (402.00 ± 7.49 beats/min); (c) valsartan (348.00 ± 15.23 beats/min); (d) rosuvastatin (330.00 ± 14.99 beats/min); (e) valsartan + rosuvastatin (290.00 ± 11.24 beats/min). L-NAME, NG-nitro-L-arginine-methyl ester.

Table 2 Effect of 3 weeks of oral daily administration of rosuvastatin, valsartan, or their combination on plasma total nitric oxide and plasma malondialdehyde in L-NAME-induced hypertensive rats

	Parameters Mean ± SEM	
Groups	Plasma NO (μmol/l)	Plasma MDA (μmol/l/dl)
Normal (distilled water)	34.12[a,b] ± 0.68	0.292[a] ± 0.005
HT-control (L-NAME, 50 mg/kg/day)	27.33*[b] ± 0.69	0.389*[b] ± 0.007
HT-valsartan (10 mg/kg/day)	36.54[a,b] ± 0.66	0.322*[a,b] ± 0.008
HT-rosuvastatin (10 mg/kg/day)	42.87*[a,b] ± 1.15	0.299[a] ± 0.005
HT-valsartan + rosuvastatin (10 mg/kg/day/drug)	46.48*[a] ± 1.02	0.293[a] ± 0.007

Each value represents the mean value ± SEM of the number of animals in each group ($n = 10$).
HT, hypertensive; L-NAME, NG-nitro-L-arginine-methyl ester; MDA, malondialdehyde; NO, nitric oxide.
*Significantly different from the corresponding normal value at P up to 0.05.
[a]Significantly different from L-NAME hypertensive control value at P up to 0.05.
[b]Significantly different from valsartan + rosuvastatin-treated value at P up to 0.05.

L-NAME for 3 weeks induced a significant acceleration in the heart rate (415.00 ± 7.49) compared with the normal value.

In contrast, oral administration of valsartan (10 mg/kg) or rosuvastain (10 mg/kg) for 3 weeks exerted a significant decrease in heart rate by 18.55 and 21.44%, respectively, compared with the HT value. Moreover, concurrent administration of valsartan and rosuvastatin normalized the heart rate of the L-NAME-induced HT rats.

Plasma nitric oxide level

After 3 weeks of daily oral administration of L-NAME (50 mg/kg), NO-deficient rats showed a significant decrease in total plasma nitrate + nitrite concentration by 19.90%. These results are presented in Table 2. Daily oral treatment with valsartan (10 mg/kg) for 3 weeks restored the plasma NO to its normal level in L-NAME HT rats. In addition, oral treatment with rosuvastatin (10 mg/kg) significantly increased the plasma NO level by 25.64% compared with the normal value. Combined treatment with rosuvastatin and valsartan acted synergistically to increase the plasma NO in L-NAME HT rats by 36.22% compared with the normal value.

Plasma malondialdehyde level

The normal value of plasma MDA was 0.292 ± 0.005 μm/dl (Table 2). Daily oral administration of L-NAME (50 mg/kg/day) for 3 weeks induced a significant increase in plasma MDA level (0.389 ± 0.007 μm/dl) compared with the corresponding normal value. Three weeks of daily oral treatment with valsartan ameliorated the increase in plasma MDA level in L-NAME HT rats. Treatment with either rosuvastatin or concurrent treatment with rosuvastatin + valsartan normalized the plasma MDA level in NO-deficient HT rats.

Plasma lipid profile

Table 3 shows that following 3 weeks of daily gavage of L-NAME (50 mg/kg/day), NO-deficient rats exhibited an insignificant effect on plasma total cholesterol, triglyceride, HDL, and LDL levels. Furthermore, a similar effect was obtained in groups of HT animals treated with either rosuvastatin, valsartan, or their combination, wherein the results did not show any significant changes in plasma lipid profile values compared with the corresponding normal values.

Body weight, heart weight, and heart weight/body weight ratio

Data presented in Table 4 reveal that no significant changes were recorded for body weight and heart weight following 3 weeks of oral administration of L-NAME (50 mg/kg/day) compared with the corresponding normal value. The ratio of heart weight/body weight of the HT rats showed a significantly higher value compared with the normal ratio. Treatment with rosuvastatin (10 mg/kg), valsartan (10 mg/kg), or their combination normalized the heart weight/body weight ratio.

Discussion

Long-term NO deficiency by administration of L-NAME extensively produced a persistent increase in ABP [6,29,30]. In the present investigation, administration of L-NAME induced a significant increase in SBP, DBP, and MABP levels of 180.54, 216.82, and 201.06%, respectively, of normal values at the end of the experimental period of 3 weeks. This elevation of ABP paralleled a decrease in the NO plasma metabolites, indicating decreased biosynthesis or availability [31,32] and a consequent increase in plasma

Table 3 Effect of 3 weeks of daily oral administration of rosuvastatin, valsartan, or their combination on total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol levels in L-NAME-induced hypertensive male rats

	Parameters Mean ± SEM			
Groups	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-cholesterol (mg/dl)	LDL-cholesterol (mg/dl)
Normal (distilled water)	67.95 ± 1.17	120.61 ± 0.75	23.75 ± 0.62	20.12 ± 1.35
HT-control (L-NAME, 50 mg/kg/day)	69.15 ± 1.08	121.89 ± 1.12	23.46 ± 0.52	21.26 ± 1.03
HT-valsartan (10 mg/kg/day)	68.98 ± 1.11	123.03 ± 0.44	23.61 ± 0.49	20.72 ± 0.78
HT-rosuvastatin (10 mg/kg/day)	68.67 ± 1.07	122.43 ± 0.99	23.44 ± 0.33	20.71 ± 0.84
HT-valsartan + rosuvastatin (10 mg/kg/day/drug)	67.46 ± 1.08	123.52 ± 1.02	22.90 ± 0.47	19.80 ± 1.19

Each value represents the mean value ± SEM of the number of animals in each group ($n = 10$).
HT, hypertensive; L-NAME, NG-nitro-L-arginine-methyl ester.

Table 4 Effect of 3 weeks of oral daily administration of rosuvastatin, valsartan, or their combination on body weight, heart weight, and heart weight/body weight ratio in L-NAME-induced hypertensive rats

	Parameters Mean ± SEM		
Groups	Body weight (g)	Heart weight (g)	Heart weight/body weight (mg/g)
Normal (distilled water)	228 ± 8.98	0.712 ± 0.028	3.122[a] ± 0.022
HT-control (L-NAME, 50 mg/kg/day)	224 ± 6.89	0.749 ± 0.028	3.343*[b] ± 0.070
HT-valsartan (10 mg/kg/day)	240 ± 5.80	0.737 ± 0.019	3.070[a] ± 0.040
HT-rosuvastatin (10 mg/kg/day)	229 ± 9.03	0.725 ± 0.021	3.165[ab] ± 0.048
HT-valsartan + rosuvastatin (10 mg/kg/day/drug)	241 ± 7.58	0.717 ± 0.017	2.975[a] ± 0.062

Each value represents the mean value ± SEM of the number of animals in each group ($n = 10$).
HT, hypertensive; L-NAME, NG-nitro-L-arginine-methyl ester.
*Significantly different from the corresponding normal value at P up to 0.05.
[a]Significantly different from L-NAME hypertensive control value at P up to 0.05.
[b]Significantly different from valsartan + rosuvastatin-treated value at P up to 0.05.

MDA [33]. This is in agreement with the results of previous studies that revealed that NO synthesis is actually reduced in animals chronically treated with L-NAME and showing vascular hypertrophy [34].

The excessive production of reactive oxygen species (ROS) was proposed to be a major factor in mediating hypertension [35]. In the L-NAME-induced hypertension model, it was suggested that a large quantity of superoxide production suppressed NO bioavailability [36,37]. Presence of oxidative stress was indicated by an increase in plasma MDA in L-NAME hypertension. This result was similar with a previous one that reported an increase in plasma and liver MDA levels in L-NAME HT rats, indicating the involvement of oxidative stress in this animal model [33].

The observed reduction in NO availability may well be explained by the efficiency of L-NAME as a potent nonspecific inhibitor of nitric oxide synthase, the enzyme responsible for synthesis of this bioactive molecule. Also, an enhanced production of ROS has been demonstrated in the model of hypertension [38] with superoxide anion (O_2^-) being an extremely rapid reactor with NO [39]. This may further explain the decreased bioavailability of NO by any overproduction of O_2^-, which removes and counteracts the relaxing activity of NO. Collectively, it has been suggested that chronic NO deficiency-induced hypertension is partly or entirely due to amplification and/or activation of other vasoconstrictor tones [30].

Data of the present study revealed that oral administration of L-NAME induced marked acceleration in heart rate. This increase in heart rate could be attributed to many postulations as described by Da Silva *et al.* [40], in whose study nitric oxide synthase inhibitors facilitated baroreceptor resetting in anesthetized rats. This may suggest that NO may participate in the homeostasis of baroreceptor function. In addition, Souza *et al.* [41] showed that hypertension induced by chronic administration of L-NAME is associated with tachycardia, increased sympathetic drive to the heart, and attenuation of the baroreflex control of the heart rate as well as the cardiac sympathetic overactivity that was associated with a decreased baroreflex sensitivity in L-NAME-induced HT rats.

The present results showed that L-NAME treatment did not alter total plasma cholesterol, triglyceride, HDL, and LDL levels. Similar results have been reported by Bouriquet *et al.* [42].

Results of the present study, which revealed that 3 weeks of valsartan (10 mg/kg/day) therapy significantly lowered the SBP, DBP, and MABP levels in L-NAME-induced HT rats ameliorated the effect of L-NAME on heart rate and normalized the heart weight/body weight ratio. These results are in agreement with the results of Amann *et al.* [43], who reported that the SBP-lowering effect of ARB was associated with an increase in circulating NO levels.

This decrease in ABP was associated with an increase in plasma NO level, which was restored close to the normal

value. In addition, a significant decrease in MDA level was observed with a nonsignificant change in the lipid profile, supporting previous evidence that angiotensin II type 1 receptor blocker could diminish the intracellular production of superoxide anions through reduced activity of angiotensin II-dependent oxidases in the endothelium and vascular smooth muscles [44,45], thus protecting NO from oxidant degradation to biologically inert or toxic molecules [46].

Furthermore, ARBs increase the basal production and release of NO independent of blood pressure reduction in essential hypertension. This suggests that they can have favorable effects on endothelial dysfunction [47].

Statins are usually used to treat hypercholesterolemia and manage patients with ischemic heart disease, although with the advent of many large clinical trials in the past 10 years their use has been extended to preventive treatment for a variety of CVDs. In the current study, rosuvastatin (10 mg/kg/day) treatment ameliorated the effect of L-NAME on SBP, DBP, and MABP levels, which may be attributed to the improved endothelial dysfunction. In addition, rosuvastatin treatment of NO-deficient HT rats ameliorated the effect of L-NAME on the heart rate and normalized heart weight/body weight ratio. Rosuvastatin improved endothelial-dependent vasodilatation in HT rats without changing the plasma cholesterol level but showed a significant decrease in plasma MDA level and was accompanied by enhanced plasma nitrite and nitrate levels reflecting the enhanced production of NO in the endothelium. Previous studies have demonstrated that HMG-CoA reductase inhibitors improve the endothelial dysfunction in HT individuals and its improvement is related to the antioxidant and anti-inflammatory effects irrespective of plasma cholesterol level [48].

Wassmann *et al.* [49] reported a significant decrease in SBP in atorvastatin-treated HT rats (204 ± 6 vs. 185 ± 5 mmHg) and reported a significant vasodilatation in the treated aortic segment. Herring *et al.* [50] observed a significant reduction in resting heart rate following pravastatin treatment. Moreover, previous studies reported that simvastatin treatment normalized muscle sympathetic nerve activity, baroreflex function, and plasma catecholamines [51] and improved left ventricular systolic function [52].

The study by Chopra *et al.* [53] revealed that systemic therapy with statins attenuates the progression of atherosclerosis by limiting endothelial injury and dysfunction, and three main mechanisms have been postulated for this effect: promotion of endothelial NO synthesis; decrease in the production of ROS and subsequent oxidative vascular stress; and direct macroscopic effects on the arterial wall. Moreover, a significantly diminished AT1 receptor expression in the vessel wall of statin-treated rats was noted, in which the AT1 receptor has historically been associated with vasoconstriction and is thus closely related to blood pressure regulation [54].

This present study revealed that 3 weeks of daily gavage of rosuvastatin (10 mg/kg/day) combined with valsartan (10 mg/kg/day) normalized SBP and ameliorated the effect of L-NAME on DBP, MABP, and heart rate in NO-deficient HT rats. In addition, the combined treatment increased the plasma NO level, suggesting that they can improve the endothelial function. The combined treatment reduced the oxidative stress marker as it normalized the plasma MDA level in L-NAME HT rats to a greater extent than did monotherapy with each drug. These beneficial effects of combined statins with RAS blockades on ABP, heart rate, endothelial function, and oxidative stress were confirmed in NO-deficient HT rats. Horiuchi *et al.* [55] tested whether statins may enhance the effect of an ARB to improve vascular remodeling in a mouse vascular injury model. They demonstrated that a combination of low-dose valsartan and low-dose fluvastatin acted synergistically to attenuate neointimal formation at doses that were without effect when administrated alone and were devoid of any effects on blood pressure and cholesterol levels.

Conclusion

In conclusion, this study suggests that coadministration of rosuvastatin and valsartan in L-NAME HT rats ameliorated the increase in the ABP and restored heart rate to its normal value. Furthermore, the combined treatment acted synergistically to increase the total plasma NO level and it also restored the plasma MDA level to its normal value. These results explain why administering rosuvastatin and valsartan could produce synergistic effects against CVDs.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

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