ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Beneficial Effects of Valsartan on Renovascular Hypertension in Rats via Nitric Oxide Preserving and Antioxidant Effects

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Manuscript Info

Manuscript History:

Received: 12 April 2014
Final Accepted: 25 May 2014
Published Online: June 2014

Key words:
valsartan; renovascular hypertension; lipid profile; antioxidant; nitric oxide

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Abstract

The present study was carried out to investigate the effects of valsartan, an angiotensin type 1 (AT_1) receptor blocker, on lipid profile, nitric oxide and antioxidant parameters in renovascular hypertension induced in rats by clipping the left renal artery. Sham-operated rats served as control. Four weeks after left renal artery ligation, hypertensive rats were treated with valsartan (5mg/kg/day, oral gavage) for 45 days. Compared with untreated hypertensive rats, valsartan treatment in hypertensive rats significantly reduced blood pressure with non significant change in serum levels of cholesterol, triglyceride, low density lipoprotein cholesterol and high density lipoprotein cholesterol. Valsartan increased significantly serum nitric oxide levels and prevented the decrease in glutathione content and superoxide dismutase enzyme activity in the aorta in treated hypertensive rats compared with untreated hypertensive group. These results suggest that valsartan in addition to its blood pressure–lowering properties, may also have beneficial effects on antioxidant parameters and endothelial function in experimental model of renovascular hypertension.

Introduction

Hypertension still retains its importance as a serious societal health problem. Hypertension has been accepted as one of the most important modifiable risk factors contributing to an increased risk of coronary artery disease and it can also damage the structure and function of organs when it is not controlled well (Bostan et al., 2011).

Hypertension is a disease in which the systemic arteries are both structurally and functionally abnormal (Raij, 1991). Apart from structural changes, the arterial wall also exhibits functional abnormalities such as impaired nitric oxide bioactivity or endothelial dysfunction (Panza et al., 1990). The endothelium is a dynamic organ with a complex structure that can secrete and synthesize vasodilator and vasoconstrictor substances in response to environmental stimulation. In endothelial dysfunction, the balance of vasoactive substances is damaged and complications occur with the vascular structure and function (Cai and Harrison, 2000). Consequently, by causing an increase in vascular resistance and damage, endothelial dysfunction has an important role in vascular pathogenesis, which is responsible for hypertension (Kırkpantur and Altun, 2006) and the presence of endothelial dysfunction conveys an adverse cardiac prognosis (Perticone et al., 2001). As a result, the decision to use drugs for antihypertensive treatment, thus bringing about a decrease in blood pressure and an improved efficiency of endothelial functions, is a rational approach.

Recently oxidative stress has been proposed as the cause of hypertension. An imbalance in superoxide and nitric oxide production may account for reduced vasodilation, which in turn can favor the development of hypertension (Ceriello, 2008).

Dyslipidemia, a strong predictor of cardiovascular disease, causes endothelial damage and the loss of physiological vasomotor activity that results from endothelial damage may become manifested as increased blood pressure (Nickenig and Harrison, 2002).

The renin-angiotensin system (RAS) has an important role in the development and maintenance of hypertension. Angiotensin II (Ang II), a member of the RAS, not only causes vasoconstriction but also regulates cytological characteristics of vascular smooth muscle cells leading to vascular remodeling (Naftilan, 1992). Thus inhibition of the RAS with either angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (ARBs) has been shown to be effective in lowering blood pressure and reducing cardiovascular mortality and morbidity.

There are currently many drugs affecting a multitude of different mechanisms in hypertension treatment. Antihypertensive drugs not only reduce blood pressure but also have a role in metabolic movements (Atacan et al., 2013). Valsartan is a highly selective, orally available antagonist of the angiotensin Type 1 (AT_1) receptor. The efficacy, tolerability and safety of valsartan have been demonstrated in hypertension, heart failure and post-myocardial infarction studies (Black et al., 2009).

Renovascular hypertension [Goldblatt two-kidney, one-clip (2K1C)] is similar to human renovascular hypertension in many aspects including endothelial dysfunction and increased oxidative stress (Basso and Terragno, 2001). Therefore, the present study was designed to assess the effect of valsartan on serum nitric oxide (NO) and lipid profile levels in renovascular hypertension induced in rats by ligation of left renal artery. The effect of valsartan on aortic antioxidant parameters as reduced glutathione (GSH) content and superoxide dismutase (SOD) enzyme activity was also assessed.

Material and Methods

Experimental Protocol

All experimental procedures were performed after approval from the ethics committee of the National Research Centre Cairo, Egypt and in accordance with the recommendations for the proper care and use of laboratory animals (Canadian Council on Animal Care Guidelines, 1993). A total of 40 male Wistar albino rats (200–250 g) obtained from the animal facility of Faculty of Medicine, Cairo University, were housed in cages, with free access to rat chow and tap water, and maintained in a temperature-controlled environment (23°C) on a 12-h light/dark cycle.

Renovascular Hypertension Model

The animals were anaesthetized with single intraperitoneal injection of thiopental sodium (40 mg/kg) (Bearnes and Eltherington, 1964). Renovascular hypertension (2K1C) was induced in rats by complete left renal artery ligation with 4-0 sterile surgical silk through a flank incision according to the method described by Cangiano et al. (1979). The contralateral kidney was left intact. Sham operated rats served as controls. They were submitted to the same surgical procedure but with no renal artery occlusion.

Drug used

Valsartan (Novartis, Pharma, Egypt) was supplied as white powder soluble in 0.5% carboxy methyl cellulose (CMC) sodium (Na) solution, freshly prepared as aqueous solution to be given as a single daily oral dose of 5 mg/kg/day (Chow et al., 1995).

Experimental design

The animals were divided into four groups as follows:

Group I: Normal control group (n=10)

Group II: Sham operated group (n= 10): Rats were surgically manipulated without ligation of renal artery and were given 0.5% CMC-Na solution by oral gavages throughout the experiment.

Group III: Hypertensive group (n=10): Rats in this group undergone ligation of left renal artery and were given 0.5% CMC-Na solution by oral gavages throughout the experiment.

Group IV: Hypertension +valsartan treated group (n=10): Rats in this group undergone ligation of left renal artery then after 4 weeks of the 2K1C procedure, to ensure that blood pressure has established, rats were treated with valsartan (5 mg/kg/day) given orally for 45 days.

At the end of the experiment (10 weeks), blood samples were collected from rat tail vein in overnight (12-h) fasting animals, after measuring the blood pressure in the various groups. Serum was separated by centrifugation at 3000 rpm for 20 min. and then clear serum was obtained and divided into three aliquots, which were then stored at -20 ℃ for determination of various biochemical parameters. All rats were sacrificed by decapitation and their aortas were removed, washed with saline and were kept in foil paper and frozen at -70 ℃ until assayed.

Measurement of blood pressure

The mean arterial pressure (MAP) was measured at the end of the experiment, 10 weeks after clipping, in all groups according to the method of Burden et al. (1979). The rats were anaesthetized with urethane (ethylcarbamate) in a dose of 1.75-2.0 gm/kg body weight injected intraperitoneally as 25% freshly prepared aqueous solution (Iwamoto et al., 1987). A polyethylene arterial cannula filled with heparinized saline was placed in the left common carotid artery and connected to the pressure transducer (Bioscience, USA) in anaesthetized rats.

Biochemical analysis

Serum cholesterol, triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) levels were quantified enzymatically using commercially available kits (Biodiagnostic kit, Egypt) according to Allain et al. (1974), Fassati and Prencipe (1982); and Lopez-Virella et al. (1977), respectively. Serum low density lipoprotein cholesterol (LDL-C) level was calculated from the formula [LDL-C= Total cholesterol - (HDL-C+TGs/5)] as described by Friedewald et al. (1972). The content of GSH and SOD enzyme activity were determined in the aortic tissue homogenate by colorimetric method (Biodiagnostic kit, Egypt) as described by Beutler et al. (1963) and Nishikimi et al. (1972) respectively. Serum NO was determined according to the method of Montgomery and Dymock (1961) by colorimetric determination of nitrite as an indicator of NO production using Biodiagnostic kit, Egypt.

Statistical analysis

The data were expressed as means ± standard error of the means (SEM). Analysis of variance (ANOVA) was performed on the means to determine whether there were significant ($p< 0.05$) differences among the groups. When ANOVA indicated statistical significance, Tukey-Kramer's multiple comparison test follows up, for intergroup comparisons. SPSS version 12 (Chicago, Illinois, USA) was used for all statistical analysis. The results were considered significant when p value <0.05.

Results

There were no statistical significant difference ($p>0.05$) in MAP, serum levels of lipid and NO as well as aortic antioxidant parameters (GSH content and SOD enzyme activity) in sham operated group as compared to control group (Table, 1).

Effect of valsartan treatment on MAP in 2K1C hypertensive rats

In the present study, the MAP of the 2K1C hypertensive rats (group III) increased to 151.76 ±7.46 mmHg at 10 weeks after clipping of the left renal artery and these values were significantly higher ($p<0.001$) than that for sham operated rats (102.22±1.85 mmHg, table, 1).

Daily oral treatment of hypertensive rats with valsartan (group IV) for 45 days induced a significant decrease ($p<0.001$) in MAP by 57% as compared to 2K1C untreated hypertensive group (65.00±4.28 vs. 151.76±7.64 mmHg respectively, table, 1). There was also significant decrease ($p<0.001$) in MAP in hypertensive rats treated with valsartan (group IV) as compared to sham operated group (65.00±4.28 vs102.22±1.85 mmHg respectively, table, 1).

Effect of valsartan treatment on lipid profile in 2K1C hypertensive rats

The present study showed significant increase ($p<0.001$) in serum cholesterol, TG, LDL-C levels with significant decrease ($p<0.001$) in serum HDL-C level in 2K1C hypertensive group (group III) as compared to sham operated group (table, 1).

However in the present study, valsartan treatment in 2K1C hypertensive rats (group IV) produced non significant change ($p>0.05$) in all lipid profile as compared to 2K1C untreated hypertensive group (group III, table, 1).

Effect of valsartan treatment on aortic antioxidant parameters in 2K1C hypertensive rats

The present study showed significant decrease ($p<0.001$) in aortic antioxidant GSH content and SOD enzyme activity in 2K1C hypertensive rats (group III) compared to sham operated group (table, 1, fig. 1).

Meanwhile, treatment of 2K1C hypertensive rats with valsartan (group IV) produced significant increase ($p<0.001$) in aortic GSH content and aortic SOD enzyme activity by 121% and 77% respectively in group IV as compared to hypertensive untreated group (table, 1, fig. 1). Valsartan treatment in hypertensive rats (group IV) normalized levels of aortic antioxidant parameters (GSH content and SOD enzyme activity) as there was non-significant difference ($p> 0.05$) in these parameters in group IV as compared to sham operated group (table, 1, fig. 1).

Effect of valsartan treatment on serum NO in 2K1C hypertensive rats

The 2K1C hypertensive rats in this study showed significant decrease ($p<0.05$) in serum NO compared with sham operated group (table, 1, fig. 2).

The 2K1C hypertensive rats treated with valsartan (group IV) showed significant increase ($p<0.05$) in serum NO by 20% compared to untreated hypertensive group (table, 1, fig. 2) with non significant difference ($p>0.05$) in serum NO in group IV compared to sham operated group (table, 1, fig. 2).

Table (1): Effect of valsartan treatment on MAP, serum levels of lipid and NO as well as aortic antioxidant parameters in 2K1C hypertensive rats

Parameters	Control (Group I)	Sham operated (Group II)	Hypertensive (Group III)	Hypertensive+valsartan (Group IV)
MAP(mmHg)	96.66 ±3.22	102.22±1.85	151.76±7.46*	65.00±4.28#*
Serum Cholesterol (mg/dl)	71.91± 6.68	62.30±5.51	109.99±8.59*	105.34±6.01
Serum TGs (mg/dl)	19.25± 1.33	21.11±0.89	38.48±2.37*	34.78±1.93
Serum LDL-C (mg/dl)	27.57± 1.78	21.80±2.07	86.98±7.72*	81.77±5.65
Serum HDL-C	37.80 ±2.69	40.36±1.19	16.89±1.34*	16.60±0.98

(mg/dl)				
Aortic GSH (nmol/gm tissue)	55.98±2.15	51.19±2.52	25.84±1.23*	57.21±2.78#
Aortic SOD (U/gm tissue)	62.178±1.47	57.77±1.26	35.38±1.27*	62.78±1.74#
Serum NO (µmol/L)	23.7±1.3	24.76±0.62	20.15±0.48**	24.14±1.44@

Values are presented as means ±SEM (n=10/group). **MAP:** mean arterial blood pressure, **NO:** nitric oxide, **2K1C**: two kidneys-one clip, **TGs:** triglyceride, **LDL-C:** low density lipoprotein cholesterol, **HDL-C:** high density lipoprotein cholesterol, **GSH:** reduced glutathione, **SOD:** superoxide dismutase

* p<0.001, * * p<0.05: significantly different from sham operated group.

# p<0.001, @ p<0.05: significantly different from hypertensive group.

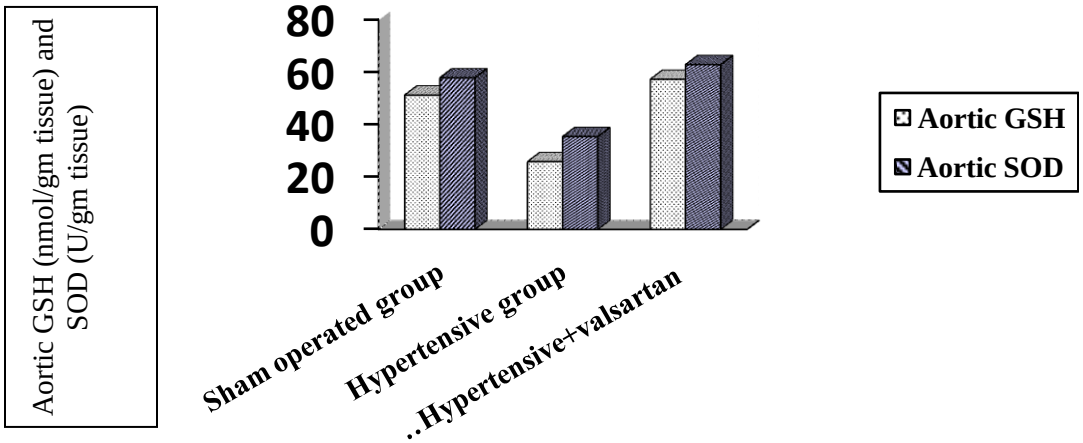

Fig. 1: Effect of valsartan on aortic antioxidant parameter in 2K1C hypertensive rats.

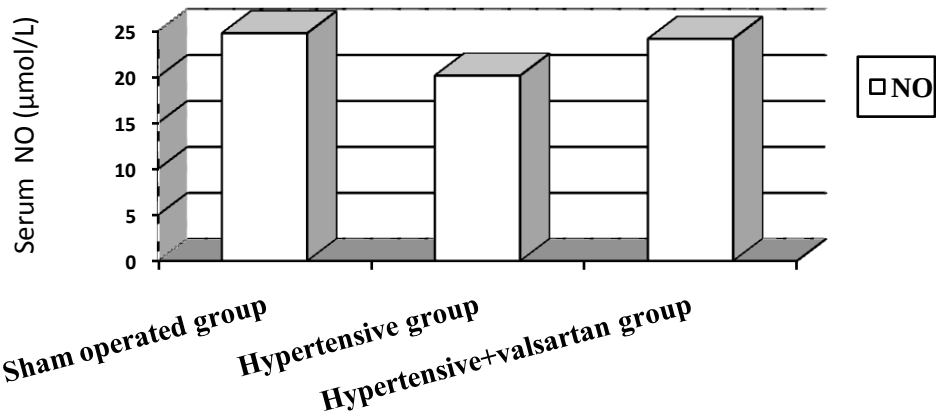

Fig. 2: Effect of valsartan on serum NO in 2K1C hypertensive rats.

Discussion

The present work showed that induction of renovascular hypertension in rats led to significant increase in MAP, and serum lipid levels together with significant decrease in serum levels of NO and aortic antioxidant parameters 10 weeks after clipping of left renal artery in 2K1C hypertensive rats as compared to sham operated rats.

Renovascular hypertension induced by 2K1C is a RAS-dependent model, leading to intrarenal vascular rarefaction and renal failure (Chade et al., 2006). Previous studies showed that the increase in circulating levels of Ang II in the 2K1C model produces sodium retention, blood volume expansion and increased systemic vascular resistance, as well as increased production of aldosterone and antidiuretic hormone, activation of the sympathetic nervous system, proliferation, fibrosis and renal injury, all of which lead to increased arterial pressure (Navar et al., 2011). Indeed, Ang II activates the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, which produce superoxide, alter the bioavailability of NO and consequently cause greater oxidative stress and inflammation, contributing to endothelial dysfunction and vascular remodeling (Oliveira-Sales et al., 2009 and 2010).

Angiotensin II acting via the AT_1 receptor has a direct vasocontrictive effect (Touyz et al., 1999): it promotes endothelin synthesis and release from the endothelial cells, thus causing vasoconstriction, and reduces NO release, thus increasing peripheral vascular resistance (Fyhrquist et al., 1995). In addition, Ang II enhances NO inactivation via an increase in oxidative stress through NADP/NADPH activation (Griendling et al., 1994). Thus, AT_1 receptor activation limits NO bioactivity both by reducing NO release and by increasing NO inactivation.

This study showed that hypertensive rats treated with valsartan for 45 days showed significant reduction in blood pressure but with non significant change in all lipid profile levels in 2K1C hypertensive treated rats as compared to untreated hypertensive rats. A study by Hanefeld and Abletshauser (2001) on the effect of valsartan treatment for 12 weeks on lipid profile in patients with mild-to-moderate hypertension showed that valsartan decreased significantly TC and LDL-C levels but did not cause any significant changes in HDL-C, VLDL-C or triglyceride levels. On the contrary to our results Kyvelou et al. (2006), found significant reduction in total cholesterol, LDL-C and TG levels with significant increase in HDL levels after six months' treatment with ARB, valsartan, in patients with essential hypertension. Meanwhile in this study, the 2K1C hypertensive rats were treated by valsartan for 45 days (6 weeks) and this is a short duration as compared to previous studies in literature. Thus this may explain the non-significant change in serum lipid profile in 2K1C hypertensive rats treated with valsartan as compared to hypertensive group.

In the present work, valsartan treatment increased significantly aortic antioxidant parameters in 2K1C hypertensive rats compared to untreated hypertensive rats. This suggests an antioxidant activity of valsartan which may be due to reduction of Ang II induces reactive oxygen species generation. In support of this mechanism, Aslam and colleagues (2006) have shown that valsartan therapy significantly reduced plasma oxidative markers in hypertensive patients with end-stage renal disease. Similarly, Hirooka et al. (2008) have recently shown that valsartan therapy improved large-artery endothelial dysfunction by modulation of oxidative stress, evidenced as reduction of urinary excretion of metabolites directly involved in oxidative stress.

The present study showed that valsartan treatment increased significantly serum NO in 2K1C hypertensive treated rats compared to untreated hypertensive rats. Our study confirms previous reports of the beneficial effect of AT_1 receptor blockade on vascular endothelium in those patients with documented atherosclerosis (Prasad et al., 2000). Another study by Schiffrin and colleagues (2000) has shown that long-term treatment with another AT_1 receptor blocker, losartan, improved both structural and functional properties of small resistance arteries in patients with essential hypertension. A study by Klingbeil et al. (2002) have shown in a carefully conducted double-blind, placebo controlled study that 6 weeks of oral valsartan therapy also favorably affected basal NO but had no effect on stimulated NO.

The increase in serum NO levels observed with valsartan in this study may be due to blockade of Ang II acting via the AT_1 receptor since Ang II limits NO bioactivity both by reducing NO release and by increasing NO inactivation via an increase in oxidative stress (Griendling et al., 1994). Oxidative stress affects NO bioactivity by reducing overall availability of locally released NO, both by accelerating NO deactivation and by reducing the endothelial nitric oxide synthase (eNOS) precursors and cofactors such as BH4 and arginine (Paolocci et al., 2001).

A major weapon of endothelial cells to fight vascular disease is eNOS, an enzyme that generates the vasoprotective molecule NO. Superoxide ($O_2\cdot^-$) reacts avidly with vascular NO to form peroxynitrite ($ONOO^-$). The cofactor BH_4 is highly sensitive to oxidation by $ONOO^-$. Diminished levels of BH_4 promote $O_2\cdot^-$ production by eNOS (referred to as eNOS uncoupling) and transformation of eNOS from a protective enzyme to a contributor to oxidative stress (Förstermann and Münzel, 2006).

In **conclusion**, the results of the present work suggest that valsartan, an AT1 receptor antagonist, in addition to its blood pressure–lowering properties, may also have beneficial effects on antioxidant parameters and endothelial function in experimental model of renovascular hypertension. Thus, valsartan may offer superior vascular protection in hypertension.

Acknowledgments

We express our gratitude to Novartis Pharma, Egypt for providing us with valsartan as a gift.

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