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Effects of Atorvastatin on the Hypertension-Induced Oxidative Stress in the Rat Brain

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ABSTRACT

Background: It is well known that the development of brain oxidative stress is one of the most serious complications of arterial hypertension that evokes brain tissue damage. The aim of this study was to examine the effects of atorvastatin treatment (20 mg/kg/day), as an antioxidant, to prevent the brain tissue oxidative stress in the hypertensive (HTN) rats. **Methods:** Experiments were performed in four groups of rats (n = 5 each group): sham, sham-treated, HTN and HTN treated. Rats were made HTN by aortic constriction above the renal arteries. After 30 days, rats were slaughtered under deep anesthesia to remove brain hemispheres. After tissue homogenization, enzyme activities of superoxide dismutase (SOD) and catalase (CAT), as well as glutathione (GSH) content and malondialdehyde (MDA) level were determined by biochemical methods. **Results:** In HTN rats, arterial blood pressure was increased about 40% and brain enzyme activities of SOD and CAT were significantly decreased compared with sham group. Induction of hypertension significantly decreased GSH content and increased MDA level of brain tissue. Treatment with atorvastatin enhanced the activity of SOD and prevented from GSH decrement during hypertension. **Conclusion:** Based on the findings of this study, treatment with atorvastatin might have saved the brain tissue of HTN rats from hypertension-induced oxidative stress. *Iran. Biomed. J. 17 (3): 152-157, 2013*

Keywords: Atorvastatin, Aortic coarctation, Oxidative stress, Hypertension

INTRODUCTION

Arterial hypertension is recognized as one of the neurodegenerative conditions that affects physiological function of brain. It is also associated with mild forms of brain tissue damage [1]. There is general consensus that the development of brain oxidative stress is one of the most serious complications of arterial hypertension that evokes vascular and brain tissue damage [2]. Increasing reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals lead to oxidation of membrane phospholipids, protein and DNA damage [3, 4]. Under physiological conditions, scavenging enzymes, including superoxide dismutase (SOD), catalase (CAT) and other non-enzymatic antioxidant systems such as glutathione (GSH) ensure the normal function of brain by inhibition of ROS toxic effects [5]. Decreased anti-oxidant capacity could aggravate ROS accumulation in the brain during hypertension [6]. Since the ability of the anti-oxidant defense system against ROS is feeble in the brain compared to other tissues; hence, the brain cells are vulnerable to death against free radicals [5]. Therefore, we have proposed that inhibition of ROS production may save the normal function and structure of brain cells.

Recent studies have shown that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, statins, may have beneficial pleiotropic effects in some pathological conditions independent of lipid-lowering effects [7, 8]. Based on the meta-analyses of clinical trials, statins may have protective effects and pretreatment with them are associated with relative risk reduction in ischemic stroke and better stroke outcomes [9]. Recent studies have demonstrated that beneficial pleiotropic mechanisms of statins involve anti-inflammatory, anti-thrombotic and anti-oxidant effects [10]. Many researchers have focused on the rapid and strong oxidative stress-lowering effects of statins [7, 11], and among them atorvastatin has a power anti-oxidant effects than others [12]. Based on the beneficial pleiotropic effects of statins, the aim of

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this study was to evaluate the effects of treatment with atorvastatin on the brain oxidative stress during aortic constricted-induced hypertension in rat.

MATERIALS AND METHODS

Male normal Wistar rats (280-300 g) were obtained from Animal House Facility of Baqiyatallah University of Medical Sciences (Tehran, Iran). All the protocols of the study were approved by the Institutional Animal Ethics Committee of Baqiyatallah University of Medical Sciences, which follows the NIH guidelines for care and use of animals. Animals were housed in standard cages in a room with controlled temperature (22-24°C), humidity (40-60%) and a light period (07.00-19.00), while having access to food and water ad libitum.

Induction of hypertension. Arterial hypertension was induced by abdominal aortic coarctation procedure [13]. In brief, under general anesthesia with ketamine (80 mg/kg, Alfasan, Holland) and xylazine (8 mg/kg, Alfasan, Holland), the abdomen was opened and abdominal aorta was surgically dissected from the inferior vena cava at slightly above the renal arteries. A blunt needle was placed alongside the isolated aorta, both were gently tightened with a stitch (3/0 silk tread), and then the needle was removed accordingly. The results of pilot experiments indicated that a 23-gauge needle size produced severe aortic constriction and raised blood pressure of the pre-narrowed segment (carotid pressure) of the aorta 30-40% higher than normal. Finally, the abdomen was sutured and after recovery, the animals were kept in separate cages for 30 days during which they had access to regular rat chow and water ad libitum. Arterial hypertension was assessed under anesthesia and via a cannulation of the right common carotid artery.

Experimental protocol and groups. The rats were randomly divided into four groups of equal numbers (n = 5). Sham-operated rats (sham) underwent the surgery at the left side of abdomen without being constricted to aorta. Surgery performed in the sham -treated rats (sham + atorvastatin), at the left side of abdomen without being aorta constriction. These rats were orally treated with atorvastatin (20 mg/kg/day) for 30 days. Hypertensive (HTN) rats underwent the surgery at the left side of abdomen and constriction of aorta performed for induction of hypertension. Finally, surgery performed in the HTN treated rats (HTN + atorvastatin), at the left side of abdomen, the same as HTN rats, and rats of this group was orally treated with atorvastatin (20 mg/kg/day) for 30 days.

Tissue preparation. Anesthesia was conducted with an i.p. injection of sodium pentobarbital (30 mg/kg, Sigma, St. Louis, MO) ketamine (40 mg/kg). The neck was opened with a ventral midline incision and a tracheotomy was performed. The rats were artificially ventilated with room air enriched with oxygen at a rate of 80 breaths/min and tidal volume of 1 ml/100 g to maintain blood partial pressure of oxygen, partial pressure of CO_2 and pH in the normal physiological ranges. A catheter was inserted into the left carotid artery for monitoring of blood pressure. Rats were slaughtered under deep anesthesia to remove brain hemispheres and heart. After heart weighting, the heart hypertrophy index was calculated using the heart weight/body weight ratio (g/kg). Brain was quickly removed and washed in ice-cold phosphate buffer saline (PBS) for GSH, malondialdehyde (MDA), SOD and CAT assays. Washed tissues were immediately immersed in liquid nitrogen and stored at -70°C until biochemical analysis. On the day of use, frozen tissue samples were quickly weighed and homogenized 1:10 in ice-cold PBS. The homogenates were then centrifuged at 14000 ×g at 4°C for 15 min. The supernatants were separated and used for enzyme activity assays and protein determination.

SOD activity. The activity of SOD was determined using the method described by Winterbourn *et al.* [14] based on the ability of SOD to inhibit the reduction of nitro-blue tetrazolium by superoxide. For assay, 0.067 M potassium phosphate buffer (pH 7.8) was added to 0.1 M EDTA containing 0.3 mM sodium cyanide, 1.5 mM nitro-blue tetrazolium and 0.1 ml of sample. Then, 0.12 mM riboflavin was added to each sample to initiate the reaction and was incubated for 12 min. The absorbance of samples was read on a Genesys 10 UV spectrophotometer at 560 nm for 5 minutes. The amount of enzyme required to produce 50% inhibition was taken as 1 U and results were expressed as U/mg protein.

CAT activity. CAT activity was measured in tissue homogenates by the method of Aebi [15]. Reaction mixture containing 0.85 ml potassium phosphate buffer 50 mM, pH 7.0 and 0.1 ml homogenate, was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.05 ml H_2O_2 (30 mM prepared in potassium phosphate buffer 50 mM, pH 7.0) and the decrease in absorbance was recorded for 3 min at 240 nm. Specific activity was expressed as 1 µmole H_2O_2 decomposed U/mg protein.

Determination of GSH level. GSH level was measured using the method of Tietz [16]. Cellular protein was precipitated by addition of 5%

Table 1. Representative mean arterial blood pressure (MAP) and heart hypertrophy index (heart weight/body weight, g/kg) of the aortic-banding groups (HTN and HTN + atorvastatin), sham and sham-treated rats, (n = 5 for each group)

Parameter \ Group	Sham	Sham + atorvastatin	HTN	HTN + atorvastatin
MAP (mmHg)	121.00 ± 5.00	133.00 ± 3.00	171.00 ± 9.00*	176.00 ± 4.00*
Heart hypertrophy index (g/kg)	2.67 ± 0.02	2.91 ± 0.07	3.48 ± 0.21*	3.47 ± 0.25*

All values are mean ± SEM. *Significantly different from sham group (P<0.05)

sulfosalicylic acid and removed by centrifugation at 2000 ×g for 10 min. GSH in the supernatant was assayed as follows: 100 µl of the protein-free supernatant of the cell lysate, 800 µl of 0.3 mM Na_2HPO_4 and 100 µl of 0.04% 5-5'-dithiobis[2-nitrobenzoic acid] in 0.1% sodium citrate. The absorbance of 5-5'-dithiobis[2-nitrobenzoic acid] was monitored at 412 nm for 5 min. A standard curve of GSH was performed and sensitivity of measurement was determined to be between 1 and 100 µM. The level of GSH was expressed as nmol/mg protein.

Lipid peroxidation Assay. The end product of lipid peroxidation was estimated by measuring the level of MDA according to the method of Satoh [17]. Tissue homogenate (0.5 ml) was added to 1.5 ml of 10% trichloroacetic acid, vortexed and incubated at room temperature for 10 min. Afterward, 1.5 ml of supernatant and 2 ml of thiobarbituric acid (0.67%) were added and placed in a boiling water bath in sealed tubes for 30 min. The samples were allowed to cool at room temperature. N-butanol (1.25 ml) was added, vortexed and centrifuged at 2000 ×g for 5 min. The resulting supernatant was removed and measured at 532 nm on a spectrophotometer. MDA concentrations were determined by using 1,1,3,3-tetraethoxypropane as a standard. MDA concentration was expressed as nmol/mg protein.

Protein assay. Protein concentration was estimated according to the method of Bradford [18] using BSA as a standard [18].

Statistical analysis. All values were presented as means ± SEM. Comparisons among the groups were performed by analysis of variance (ANOVA), followed by Tukey's post-hoc test, and P<0.05 was considered as statistically significance.

RESULTS

Arterial pressure and heart hypertrophy. Table 1 shows mean arterial blood pressure (MAP, mmHg) value and heart hypertrophy index at the end of experiment (day 30). The MAP averages of sham and sham-treated rats (nonaortic-constricted rats) were 121 ± 5 and 133 ± 3 mmHg, respectively without any significant difference among them. Aortic constriction enhanced MAP by >40% in aortic-constricted rats (171 ± 9 mmHg). Hence, atorvastatin treatment did not statistically alter MAP of HTN treated rats in comparison to non-treated HTN group. There was a 41% increment in heart hypertrophy index of aortic-constricted rats (HTN and HTN-treated rats). The results of treated groups indicated that the atorvastatin treatment of sham and HTN rats did not statistically alter heart hypertrophy index of these rats during 30 days of treatment.

SOD activity. Figure 1 shows SOD activity of brain tissue at the end of experiment (day 30). Obtained results indicated that aortic constriction significantly decreased the SOD activity of brain in HTN rats (P<0.05). Atorvastatin treatment of sham group did not statistically alter SOD activity of brain in these rats during 30 days of treatment, while treatment with atorvastatin in HTN rats significantly increased the brain SOD activity in comparison to non-treated HTN rats (P<0.05).

CAT activity. Figure 1 denotes the quantitative CAT activity of brain tissue in the end of experiment. The results of HTN rats (HTN and HTN treated groups) indicated that aortic constriction decreased CAT activity of brain tissue. Atorvastatin treatment of sham and HTN rats did not statically alter CAT activity of these rats during 30 days of treatment because the results of sham-treated and HTN-treated rats did not statistically differ from sham and HTN rats.

GSH content. Total GSH content of brain homogenates has been presented in Figure 2. Aortic constriction significantly decreased GSH content of brain tissue in HTN rats (P<0.05). Atorvastatin treatment of sham group did not statistically alter GSH level of brain in these rats during 30 days of treatment, while atorvastatin in the HTN-treated rats prevented significant decrease of GSH content of brain; hence, the data of this group did not statistically differ from sham group.

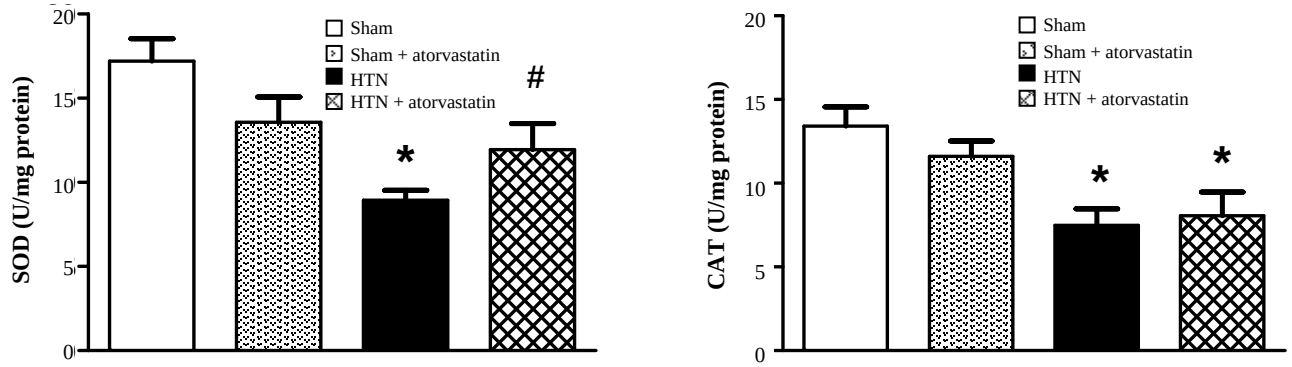

Fig. 1. Brain superoxide dismutase (SOD) and catalase (CAT) activity of the aortic-banding groups (HTN and HTN + atorvastatin), sham and sham-treated rats (n = 5 for each group). All values are mean ± SEM. * and # shows significantly different from sham and HTN groups, respectively (P<0.05).

Lipid peroxidation. Total MDA content of brain tissue, as a lipid peroxidation, has been shown in Figure 2. There was a significant increment in the total MDA content of aortic-constricted rats (HTN and HTN-treated rats) compared with sham group (P<0.05). The results of treated groups indicated that the atorvastatin treatment of sham and HTN rats did not statically alter total brain MDA content of these rats during 30 days of treatment.

DISCUSSION

It is well known that releasing of different factors and some active metabolites result in tissue damage with different unknown mechanisms during hypertension [19]. It is appear, production of oxygen free radicals (ROS) and induction of oxidative stress during hypertension are the most important mechanisms in this phenomena [6]. Using an experimental model of hypertension by severe aortic constriction above the renal arteries, we demonstrated that elevated arterial blood pressure above the stenotic site induced cardiac hypertrophy in concomitant with oxidative stress in the brain. In our study, hypertension attenuated enzymatic antioxidant systems (reduction of SOD and CAT activity), reduced brain GSH content and increased lipid peroxidation. Also, the results of this study indicated that atorvastatin treatment during HTN prevented alteration of enzymatic antioxidant systems by increasing SOD activity and GSH level.

The present study utilized abdominal aortic constriction above the renal arteries for induction of hypertension. Aortic constriction was enhanced MAP by 40% in HTN rats (Table 1). Additionally, cardiac hypertrophy index was increased by 41% in HTN rats (Table 1). Cardiac hypertrophy, in the aortic-constricted rats, is a physiological adaptation to an enhanced heart workload. Atorvastatin did not significantly change MAP and cardiac hypertrophy index of HTN group and sham treated rats (Table 1). Based on a recent study, atorvastatin reduced systolic and diastolic pressure of HTN conditions and treatment with this drug prevented development of cardiac hypertrophy partly through reducing active Ras and p44/42 MAPK [20]. In our study, atorvastatin could not induce these effects, which might be greatness of hypertension or shorter length of treatment.

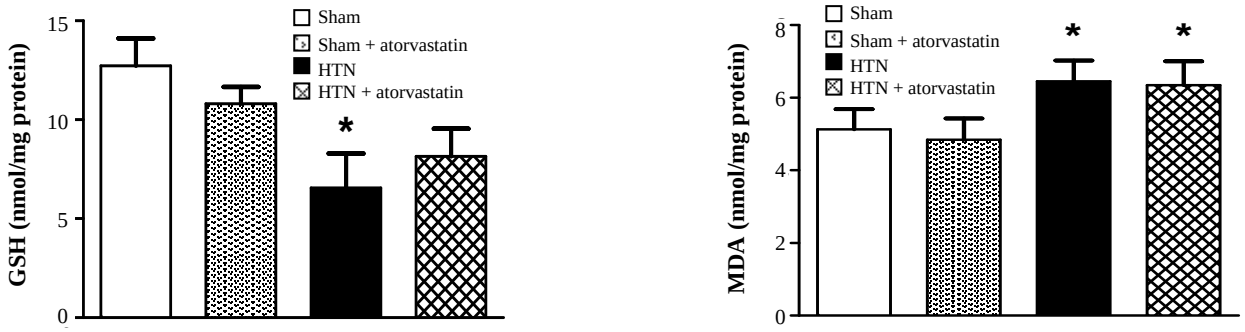

Fig. 2. Total glutathione (GSH) and total malondialdehyde (MDA) content in brain homogenates of the aortic-banding groups (HTN and HTN + atorvastatin), sham and sham-treated rats (n = 5 for each group). All values are mean ± SEM. *Significantly different from sham group (P<0.05).

In our study, the brain activity of SOD and CAT, which involved in prevention of oxidative stress, were significantly reduced in response to hypertension. Increasing of ROS production (superoxide and hydroxyl anions) during HTN can affect protein function and gene expression of some growth factors [5]. According to a recent study, increasing ROS production during pathological conditions associated with altered activity of antioxidant enzymes such as CAT and SOD [21]. Since the ability of the antioxidant defense system against ROS is feeble in the brain compared to other tissue; hence, the brain cells are vulnerable to death against free radicals [5]. Therefore, attenuation of the activity of these enzymes in some pathological conditions leads to brain oxidative stress.

In the present study, significant reduction of GSH as another marker of oxidative stress occurred in the brain of HTN rats (Fig. 2). Because GSH acts as a scavenger of free radicals, reduction of its concentration leads to ROS accumulation and development of oxidative stress in brain tissue [3]. Also in this study, the level of MDA (an indicator of lipid peroxidation) was significantly increased in the brain of HTN rats (Fig. 2). During generation of oxidative stress, the amount of MDA level increases, indicating the induction of lipid peroxidation [21]. In our study, attenuated activity of SOD and CAT, reduction of GSH, and an increase in MDA level were overwhelming reasons in development of oxidative stress in the brain of HTN rats.

Accordingly, the increased ROS production during hypertension leads to brain tissue damage. Therefore, inhibition of ROS production in the brain of HTN individual by medications can be neuroprotective effects. In our study, treatment with atorvastatin significantly enhanced the activity of SOD, which was attenuated by hypertension (Fig. 1). Since atorvastatin increased the activity of SOD, superoxide anions were rapidly dismutated by SOD to hydrogen peroxide, more stable form of ROS, which was then converted to H_2O by CAT or GSH peroxidase. Also in the present study, atorvastatin could be avoided from significant reduction of GSH content as scavenger of free radicals (Fig. 2). The results of the present study are matched with other studies that atorvastatin had protective action against oxidative stress in other tissues such as heart in certain pathological conditions [8, 11, 12]. However in the present study because of the severity of hypertension, atorvastatin did not change the decreased activity of CAT, but increased MDA level during hypertension. Perhaps, long-term treatment with high dosage of atorvastatin is required. Another interesting point in the present study is that atorvastatin does not change the antioxidant defense system of rat brain in normal circumstances.

In summary, the findings of the present study indicated that aortic constriction-induced hypertension attenuated the antioxidant defense system of brain tissue, which leads to increased ROS accumulation in the brain. Also, the results of this study demonstrated the ability of atorvastatin to reinforce antioxidant defense system in the brain of HTN rats.

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