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The anti-oxidant and anti-apoptotic effects of nebivolol and zofenopril in a model of cerebral ischemia/reperfusion in rats

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ABSTRACT

The aim of this experiment was to investigate whether nebivolol and zofenopril have protective effects against oxidative damage and apoptosis induced by cerebral ischemia/reperfusion (I/R).
There were seven groups of rats, with each containing eight rats. The groups were: the control group, I/R group, I/R plus zofenopril, I/R plus nebivolol, I/R plus nebivolol and zofenopril, zofenopril only and nebivolol only. Cerebral I/R was induced by clamping the bilateral common carotid artery and through hypotension. The rats were sacrificed 1 h after ischemia, and histopathological and biochemical analyses were carried out on their brains. The total antioxidant capacity was evaluated by using an automated and colorimetric measurement method developed by Erel. I/R produced a significant increase in the levels of total oxidant status and malondialdehyde levels, the number of caspase-3 immunopositive cells and activities of prolidase and paraoxonase in brain when compared with the control group ($p<0.05$). A significant decrease in brain total antioxidant capacity and nitric oxide levels were found in I/R group when compared with the control group ($p<0.05$). Both nebivolol and zofenopril treatment prevented decreasing of the total antioxidant capacity and nitric oxide levels, produced by I/R in the brain ($p<0.05$). Both nebivolol and zofenopril treatment prevented the total oxidant status, malondialdehyde levels, activities of paraoxonase and prolidase from increasing in brains of rats exposed to I/R ($p<0.05$).
In conclusion, both nebivolol and zofenopril protected rats from ischemia-induced brain injury. The protection may be due to the indirect prevention of oxidative stress and apoptosis.

1. Introduction

Ischemic stroke is one of the most frequent causes of death and neurological disability (Flynn et al., 2008). Effective stroke treatment requires recanalization of occluded cerebral blood vessels. However, reperfusion after cerebral ischemia can cause brain injury, leading to cerebral edema, brain hemorrhage, and neuronal death by apoptosis/necrosis. These complications, together with excessive production of reactive oxygen species (ROS) in mitochondria, significantly limit the benefits of stroke treatment (Jung et al., 2010). The reperfusion may actually potentiate central nervous system ischemic damage, mainly due to an inflammatory response involving free oxygen radicals, proteases, and activated leukocytes (Jung et al., 2010; Lapchak, 2009; Ozerol et al., 2009). Most of the potential detrimental effects of oxidative stress are considered to be due to the generation of ROS (Maes et al., 2011).

Hypertension is a major risk factor for stroke. It has been reported that moderate reductions in blood pressure during the first week after hospital admission is associated with short-term functional improvement in patients who have suffered from acute ischemic stroke. Therefore, antihypertensive drugs have been used to treat stroke patients (Rodriguez-Garcia et al., 2005). Activating renin–angiotensin–aldosterone system (RAAS) and subsequent elevating tissue angiotensin II and plasma aldosterone are crucial in the pathogenesis of hypertensive complications (Perret-Guillaume et al., 2009). Activating of the RAAS through enhanced

Abbreviations: ROS, reactive oxygen species; ACE, angiotensin converting enzyme; I/R, ischemia/reperfusion; SOD, superoxide dismutase; NO, nitric oxide; TAC, total antioxidant capacity; TOS, total oxidant status; PON-1, paraoxonase; MDA, malondialdehyde; RAAS, renin–angiotensin–aldosterone system; NOS, nitric oxide synthase.

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levels of angiotensin-II leads to an increase in oxidative stress (Garrido and Griendling, 2009). Moreover, oxidative stress is significantly reduced by treatment with angiotensin-converting enzyme (ACE) inhibitors (Cacciatore et al., 2011). The bioavailability of tissue nitric oxide (NO) appears to be a critical regulator for both the RAAS and sympathetic nervous system control of blood pressure (Ambrosioni, 2007; Ilhan et al., 2004; Toblli et al., 2010). Zofenopril is a potent ACE inhibitor. When zofenopril is used much higher than the therapeutic concentrations, it has indirect antioxidant effects by the inhibition of ACE (Altunoluk et al., 2006; Ambrosioni, 2007, Borghi et al., 2007). It has been shown that zofenopril can protect the heart and kidneys from ischemia reperfusion (I/R) damage (Altunoluk et al., 2006; Frascarelli et al., 2004). Nebivolol is a third-generation β_1 adrenergic blocker with distinct pharmacologic and pharmacodynamic properties compared with other beta blocker agents (Kamp et al., 2010). It has been demonstrated that nebivolol has protective effects in the kidneys, spinal cord and ilium against I/R injury (Gandhi et al., 2008; Ilhan et al., 2004; Soydan et al., 2011). Nebivolol therapy promotes endothelium-dependent vasodilation through the L-arginine/NO pathway (Kamp et al., 2010). Also, it has been shown that one of the mechanisms of nebivolol's antioxidant activity is due to a reduction of ROS produced by a NADPH oxidase system (Cominacini et al., 2003). Antihypertensive therapy is usually needed in patients who have suffered from ischemic stroke (Ilhan et al., 2004). Therefore, antihypertensive drugs leading to anti-oxidation and increased bioavailability of NO are likely to be used in the prevention of I/R in stroke patients.

To our knowledge, the protective effects of zofenopril and nebivolol in cerebral I/R injury have not been previously studied. Oxidative stress is probably one of the mechanisms involved in neuronal damage induced by I/R. The brain contains many antioxidant molecules that prevent and/or inhibit harmful free radical reactions (Dringen, 2007). Tissue concentrations of antioxidants can be measured one by one, but this procedure is time-consuming, labor-intensive and costly, and requires complicated techniques. On the other hand, total antioxidant capacity (TAC), whose measurement method has been recently specified and developed, can reflect the total antioxidant status of the tissues. In this method, the TAC of tissues, or sera which acts against potent free radical reactions strongly leading to oxidative damage of biomolecules such as lipids, proteins and DNA is analyzed (Erel, 2004). Sera, or the tissue levels of oxidants can be measured separately in the laboratory. However, the measurement of the total oxidant status (TOS) accurately reflects the oxidative status of plasma or sera (Alp et al., 2010; Yumru et al., 2009). A recently developed method by Erel (2005) has been used to measure the TOS of sera. TAC may be an important factor for providing protection from neurological damage caused by I/R related oxidative stress. Likewise, TOS may indicate the level of all free oxidant radicals caused by I/R related oxidative stress.

The protective effects of zofenopril and nebivolol in cerebral I/R injury have not been previously studied. In this experiment, nebivolol and zofenopril were tested on rats with cerebral I/R to deduce their protective efficacies, with note to the biochemical and histopathological outcomes.

2. Material and methods

2.1. Animals

This study was approved by Dicle University Animal Ethical Committee. Female Sprague–Dawley rats (aged 8–12 weeks) weighing 220 ± 30 g (mean ± standard deviation) obtained from Laboratory Animal Production Unit of Dicle University were used in the experiment. The rats were placed in a temperature- and humidity-controlled room, with the temperature set at 22 ± 2 °C and humidity set at 50 ± 5%). Twelve-hour light–dark cycles were maintained for 1 week prior to the experiment. The rats were fed a standard diet and tap water ad libitum.

2.2. Experimental procedure

Fifty-six rats were divided into seven groups: group I (n = 8), the control (sham surgery, no I/R) group; group II (n = 8), the I/R group (only I/R); group III (n = 8), the I/R, zofenopril-treated group (zofenopril + I/R); group IV (n = 8), the I/R nebivolol-treated group (nebivolol + I/R); group V (n = 8), the I/R, nebivolol- and zofenopril-treated group (nebivolol + zofenopril + I/R); group VI (n = 8), the zofenopril-treated group (zofenopril, no I/R); group VII (n = 8), the nebivolol treated group (nebivolol, no I/R). Before the operation, rats were anesthetized with ketamine 70 mg/kg and xylazine 10 mg/kg by intraperitoneal injection. The anesthesia was maintained until the end of the experiment (1.5 h). The cerebral ischemia was induced in the rats under general anesthesia with two vessel occlusion combined with systemic hypotension according to the method of Smith et al. (1984). Briefly, in the supine position, a midline ventral incision was made in the neck. Both common carotid arteries were exposed, separated from the vagus nerve and occluded for 30 min with microaneurysmal clamps. The clamps were then released, allowing of carotid blood flow to 60 min reperfusion. After cerebral ischemia, incisions were sutured to prevent minor bleeding. In sham-operated animals, the arteries were freed from connective tissue but were not occluded. Control, zofenopril and nebivolol groups underwent skin incision without cerebral I/R. The medications were administered before the I/R, to investigate the effects of the medications against I/R damage. Doses of nebivolol and zofenopril were measured specifically for rats. Nebivolol doses were administered (6 mg/kg/day orally) according to the study of Gschwend et al. (2009). Nebivolol (Vasoxen, Ibrahim Etem Ulagay) was given orally (6 mg/kg/day) for 5 days, starting 5 days before the cerebral I/R, to the nebivolol, nebivolol + I/R, and zofenopril + nebivolol + I/R groups. In previous ischemia reperfusion study, zofenopril has been used 15 mg/kg. Zofenopril 15 mg/kg was administered (Altunoluk et al., 2006). Zofenopril (Zoprotec, UFSA) was given orally (15 mg/kg/day) for 5 days, starting 5 days before the experiment, to the zofenopril, zofenopril + I/R, and zofenopril + nebivolol + I/R groups. In the control and I/R groups, saline was given in the same way for 5 days. Cerebral I/R was performed to groups II, III, IV and V. After the completion of the reperfusion period, the rats were sacrificed in order to undergo biochemical and histological assessment.

2.3. Biochemical analyses

The excised cerebrum samples were weighed and immediately stored at −50 °C. The cerebrum tissues were homogenized in five volumes (w/v) with 1.15% ice-cold KCl solution. Assays were performed on the supernatant of the homogenate that is prepared at 14,000 rpm for 30 min at +4 °C. The protein concentration of the tissue was measured using Lowry's et al. method (1951). Superoxide dismutase (SOD) activity was measured according to the method described by Fridovich (1974). The lipid peroxidation level in the cerebrum was expressed as malondialdehyde (MDA). It was measured according to procedure of Ohkawa et al. (1979). Nitric oxide (NO) levels were determined using the Griess method (Cortas and Wakid, 1990). Paraoxonase-1 (PON-1) activity was measured spectrophotometrically using the modified Eckerson et al. method (1983).

The TAC of supernatant fractions was measured using a novel automated and colorimetric method developed by Erel (2004). This 2,2 V-azinobis(3-ethylbenzo-thiazoline-6-sulfonate) (ABTSS+)-based method, in which a colorless molecule, reduced ABTS, is oxidized to a characteristic blue-green ABTSS+. When the colored ABTSS+ is mixed with any substance that can be oxidized, it is reduced to its original colorless ABTS form again; in contrast, the reacted substance is oxidized. This feature is the basic principle of the methods that use ABTS. The reduced ABTS molecule is oxidized to ABTSS+ using hydrogen peroxide alone in acidic medium (the acetate buffer 30 mmol/l pH 3.6). In the acetate buffer solution, the concentrate (deep green)