The protective effect of nebivolol on ischemia/reperfusion injury in rabbit spinal cord

Atilla Ilhan\textsuperscript{a,}\textsuperscript{*}, H. Ramazan Yilmaz\textsuperscript{b}, Ferah Armutcu\textsuperscript{c}, Ahmet Gurel\textsuperscript{c}, Omer Akyol\textsuperscript{d}

\textsuperscript{a}Department of Neurology, Turgut Ozal Medical Center, Inonu University Medical Faculty, Malatya 44049, Turkey
\textsuperscript{b}Department of Medical Biology and Genetics, Suleyman Demirel University, Medical Faculty, Isparta, Turkey
\textsuperscript{c}Department of Biochemistry, Kara Elmas University Medical Faculty, Zonguldak, Turkey
\textsuperscript{d}Department of Biochemistry, Inonu University Medical Faculty, Malatya, Turkey

Accepted 29 June 2004
Available online 12 September 2004

Abstract

The aim of this experimental study was to investigate whether nebivolol has protective effects against neuronal damage induced by spinal cord ischemia/reperfusion (I/R). Twenty-one rabbits were divided into three groups: group I (control, no I/R), group II (only I/R) and group III (I/R+nebivolol). Spinal cord ischemia was induced by clamping the aorta both below the left renal artery and above the aortic bifurcation. Seventy-two hours postoperatively, the motor function of the lower limbs was evaluated in each animal. The animals were sacrificed at 72 h, and histopathological and biochemical analyses were carried out in the lumbar spinal cords. The motor deficit scores in nebivolol group were different from I/R group at 72 h (3.25 ± 0.70 vs. 1.75 ± 1.28, \(p=0.01\)). I/R produced a significant increase in the superoxide dismutase (SOD), xanthine oxidase (XO), adenosine deaminase (ADA) and myeloperoxidase (MPO) activities in spinal cord tissue when compared with control group. Nebivolol treatment prevented the increase of all those enzymes activities produced by I/R. A significant decrease in spinal cord glutathione peroxidase (GSH-Px) level was seen in I/R group and nebivolol treatment prevented the decrement in the spinal cord tissue GSH-Px contents. On the other hand, I/R produced a significant increase in the spinal cord tissue malondialdehyde (MDA) and nitric oxide (NO) contents, this was prevented by nebivolol treatment. In conclusion, this study demonstrates a considerable neuroprotective effect of nebivolol on neurological, biochemical and histopathological status during periods of spinal cord I/R in rabbits.

© 2004 Elsevier Inc. All rights reserved.

Keywords: Ischemia/reperfusion injury; Nebivolol; Oxidative damage; Spinal cord

1. Introduction

Paraplegia is a serious complication sometimes resulting from surgery on the thoracic aorta, which the reported incidences range from 4% to 33% (Svensson et al., 1993). Therefore, in an attempt to prevent this complication, various methods of spinal cord protection have been suggested, including temporary shunts or partial bypass (Svensson et al., 1986a), hypothermia (Tabayashi et al., 1993), drainage of cerebrospinal fluid (Mc Cullough et al., 1988) and pharmacological measures (Wadouh et al., 1984; Svensson et al., 1986b). Despite their use, paraplegia remains a persistent complication (Zvara, 2002). Although the exact mechanism of such complication is not fully understood, it is believed that the cause of spinal cord dysfunction is ischemia from hypoperfusion during aortic cross-clamping. However, in some patients paraplegia could be developed 1–5 days later (delayed-onset paraplegia). The cause of delayed onset paraplegia has been attributed to embolization or thrombosis.
to anterior spinal artery, postoperative hypotension, deleterious effects of leukocytes and microglia, free radical production and apoptosis (Moore et al., 2000; Kirsh et al., 1992; Cacciano and Kraig, 1993). Spinal cord damage as a result of ischemia/reperfusion (I/R) is thought to involve the destructive effect of reactive oxygen species (ROS). Previously, we have shown that the administration of caffeic acid phenethyl ester (CAPE), which exhibits antioxidant properties, before aortic cross-clamping improved neurologic outcome and oxidative damage in a rabbit model for spinal cord I/R (Ilhan et al., 1999).

Nebivolol is a new selective β-adrenergic blocking agent that possesses a peculiar pharmacodynamic profile and an original chemical structure, by which it differs from traditional β-blockers. Nebivolol is endowed with peripheral vasodilating properties mediated by the modulation of the endogenous production of nitric oxide (NO), as well as additional antioxidative effects (Mangrellaa et al., 1998).

In this study, the authors investigated the protective efficacy of nebivolol, which is a β-adrenoceptor antagonistic, on the neurological and histopathological outcomes of spinal cord ischemia and studied its impact on the I/R-induced oxidative damage in our experimental settings.

2. Methods

2.1. Animals

Twenty-one New Zealand White rabbits weighing 2.4–3.2 kg (mean 2.7 kg) were used in this study. The experimental protocol was evaluated and approved by the Ethics Review Committee of Inonu University, Faculty of Medicine, prior to the study. The animals were fasted for 12 h and operated on in a room kept at 24 °C. They were anesthetized with intramuscular ketamine hydrochlorid with an initial dose of 50 mg/kg and xylazine 3 mg/kg, followed by 25 mg/kg fractionally as need during the procedure. The animals were allowed to breathe room air without mechanical ventilation. Body temperature maintained close to 38 °C using a heated operation table. An intravenous catheter (24-gauge) was placed in an ear vein and preoperatively cefazoline 10 mg/kg was administered as a single dose. Maintenance fluid of 0.9% NaCl was infused at a rate of 20 ml/h during the procedure.

2.2. Surgical technique

The animals were placed in the supine position with the pelvis partially rotated to the right. After the surgical preparation, the vertical incision was made from the left costal margin directed towards the pubis. The abdominal aorta was exposed through a retroperitoneal approach and mobilized from just inferior to the left renal vein down to the aortic bifurcation. Heparin (100 U/kg) was administered intravenously 5 min before aortic occlusion. The animals underwent aortic occlusion were divided into two groups each consisting of eight rabbits. In nebivolol group, 50 mg/kg nebivolol (racemic mixture of nebivolol, Vasoxen, Ibrahim Ethem) orally; in I/R group, a similar volume of sterile saline solution were administered orally. In the nebivolol group, the administration of nebivolol solution was started two days before from aortic cross-clamping to obtain steady blood levels. Groups of nebivolol and I/R underwent surgical procedures as described above and spinal cord ischemia was induced with clamping the aorta just below the renal vein with a bulldog clamp (FB328). A second similar clamp was placed above the aortic bifurcation for occluding iliac collateral circulation. Animals were subjected to 21 min of cross-clamp time. At the end of occlusion time, the clamps were removed and restoration of the blood flow was verified visually. Animals in sham control group (n=5) underwent a surgical procedure similar to the other groups but the aorta was not occluded. This group of animals was used for eliciting the effects of anesthesia and operation on results and also determining the biochemical parameters studied in the normal spinal cord tissue. The catheters were removed and the incisions closed. When the animals awakened from anesthesia, they were returned to their cages.

2.3. Evaluation of neurological status

Neurological status of animals was assessed blindly by neurologist at 6, 12, 24, 48 and 72 h. Crede’s maneuver was used for evacuation of the urinary bladder when necessary. Neurological status was scored by assessment of hind limb motor function deficit. A score of 0–5 was assigned to each animal, as follows: score 0, no voluntary hind limb function; score 1, movements of joints perceptible; score 2, active movement but able to sit with assistance; score 3, sit without assistance; score 4, weak hop; score 5, normal hop.

2.4. Biochemical analyses

After the last neurological examination at 72-h post-operation, the animals were anaesthetized with the use of ketamine (50 mg/kg) and blood samples were collected through a cardiac puncture. For biochemical analysis, spinal cords and serum were used in all rabbits from each group. After spinal cord tissues were obtained, all spinal cord tissues were washed two times with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (−30 °C) until processing (maximum 10 h). Tissues were homogenized in a four volumes of ice-cold Tris–HCl buffer (50 milimolar, pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T18 basic, USA) after cutting of the spinal cords into small pieces with a scissors (for 2 min at 5000 rpm). NO and malondialdehyde (MDA) concentrations as well as protein levels were carried out at this stage. The homogenate was then centrifuged at 5000×g for 60 min to remove debris. Clear upper supernatant fluid was taken and glutathione perox-
2.5. MDA determination

Tissue and serum MDA level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90–100 °C (Esterbauer and Cheeseman, 1990). In the TBA test reaction, MDA or MDA-like substances and TBA react together for production of a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 90 °C for 30 min, the clear upper layer (the ethanol phase) was taken and used in the superoxide dismutase (SOD) activity and protein assays. The measurements were made at different steps because of the fact that some analytes may undergo subsequent oxidation after advanced preparation procedures. Also, extraction procedure is needed for SOD activity measurement because of the artifact effect of supernatant on the color development. All preparation procedures were performed at +4 °C. Blood samples by cardiac puncture were drawn into potassium ethylenediaminetetraacetic acid (EDTA)-containing tubes. The blood samples were centrifuged at 1000×g for 10 min at 4 °C to remove plasma.

2.6. NO determination

As NO measurement is very difficult in biological specimens, tissue nitrite (NO$_2^-$) and nitrate (NO$_3^-$) were estimated as an index of NO production. The method for spinal cord, nitrite and nitrate levels was based on the Griess reaction (Cortas and Wakid, 1990). Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite+ nitrate) was measured after conversion of nitrate to nitrite by copperized cadmium granules by the spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10$^{-8}$–10$^{-3}$ mol/l) of sodium nitrite. Linear regression was done by using the peak area from nitrite standard. The resulting equation was then used to calculate the unknown sample concentrations. Results were expressed as micromoles per gram protein in spinal cord tissue and micromoles per liter in serum.

2.7. SOD activity determination

Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. (1988). The principle of the method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine-XO system as a superoxide generator. Activity was assessed in the ethanol phase of the serum and spinal cord homogenate after 1.0 ml ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was also expressed as units per milliliter for serum and units per milligram spinal cord protein.

2.8. XO activity determination

XO activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbency at 293 nm, according to Prajda and Weber’s method (1975). A calibration curve was constructed by using 10–50 mU/ml concentrations of standard XO solutions (Sigma X-1875). One unit of activity was defined as 1 μmol of uric acid formed per minute at 37 °C, pH 7.5, and expressed as units per milliliter in serum and units per gram protein in spinal cord homogenate.

2.9. ADA activity determination

Plasma and tissue ADA activities were estimated spectrophotometrically by the method of Giusti (1974), which is based on the direct measurements of the formation of ammonia, produced when ADA acts in excess of adenosine. Results were expressed as units per liter in serum and units per gram protein in spinal cord tissue.

2.10. GSH-Px activity determination

GSH-Px activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction in the tube, which is containing following items: nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH), sodium azide and glutathione reductase, was initiated by addition of H$_2$O$_2$ and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given as units per gram protein in spinal cord tissue. All samples were assayed in duplicate.

2.11. Myeloperoxidase (MPO) activity determination

MPO activity was determined using a 4-aminoantipyrine/phenol solution as the substrate for MPO-mediated oxidation by H$_2$O$_2$ and changes in absorbance at 510 nm (A$_{510}$) were recorded (Wei and Frenkel, 1993). One unit of MPO activity is defined as that which degrades 1 μmol H$_2$O$_2$/min.
at 25 °C. Data are presented as mU/g protein. All samples were assayed in duplicate.

2.12. Protein determinations

Protein assays were made by the method of Lowry et al. (1951).

2.13. Histopathological examination

A histopathological evaluation was carried out in the spinal cords and abdominal aorta. The entire spinal column and abdominal aorta with lumbar arteries was removed. Distal parts of the specimens were fixed in 10% buffered formalin for about 10 days before being set in paraffin blocks for sectioning. Five-micrometer sections were cut from paraffin-embedded blocks and the sections were taken to slides for hematoxylin and eosin staining (H&E). Abdominal aorta and its branches were examined for revealing possible thrombosis or embolic occlusion.

2.14. Statistical analysis

Non-parametric analyses with Mann–Whitney U-test were performed on the data of the physiological and biochemical variables. Motor deficit scores of animals were compared using repeated measures analysis of variance (ANOVA) with the statistical significance of each comparison adjusted for the multiple comparisons using a Bonferroni correction. Fisher exact probability test was used in the analysis of bladder and bowel function. Correlations among the biochemical parameters in each group were tested for with Spearman’s test. A value of \( p < 0.05 \) was considered significant. Data are expressed as mean±S.D. All statistical analyses were carried out using SPSS statistical software (SPSS for Windows, Chicago, IL).

3. Results

3.1. Physiological variables and neurological outcome

Statistical analysis between the three groups for the various physiologic variables (mean levels of blood glucose, arterial blood gases, pH and body temperatures) did not disclose differences. Also, there was no difference in the loss of bowel and bladder sphincter control between the three groups. All rabbits survived without major complications. Twenty-one minutes of ischemia resulted in severe motor deficit in the hind limbs of all animals, while all control animals maintained normal motor behavior (score of 5), as assessed by the motor deficit score (Fig. 1). Pretreatment with nebivolol did not prevent the development of paraplegia. Most of animals in nebivolol group exhibited score 3 or score 4 motor functions at 72 h. There were significant differences in motor deficit scores between control and I/R (\( p<0.0001 \)), and control and nebivolol groups (\( p=0.01 \)). On the other hand, the scores in nebivolol group were different from I/R group at 72 h (3.25±0.70 vs. 1.75±1.28, \( p=0.01 \)).

3.2. Histopathology

Histopathological photographs of sections are shown in Fig. 2. At 72 h, histopathologic analysis of the H&E-stained sections from the fourth lumbar sacral segments of the nebivolol and I/R groups revealed changes consistent with ischemic injury. The control rabbits showed no signs of neuronal damage, with many large motor neurons in anterior horn. In contrast, animals in I/R group with complete paralysis (score 0) had total destruction of the motor neurons with intensely eosinophilic cytoplasm, Nissl granule loss and pronounced vacuolization of the anterior horn, as well as by the presence of infiltrating neutrophils and mononuclear phagocytes. In nebivolol group, spinal cords of rabbits scored 3 to 4 exhibited mild degrees of destruction such as the perikaryon becoming shrunken. The scores in nebivolol group were different from I/R group at 72 h (3.25±0.70 vs. 1.75±1.28, \( p=0.01 \)). Histologic examination of abdominal aorta and its branches were normal and reveal no thrombus formation in all animals.

3.3. The biochemical analysis of oxidant stress markers in spinal cord tissue

In the I/R group, I/R produced a significant increase in the SOD, XO, ADA and MPO activities in spinal cord tissue when compared with control group (Table 1). Although the increased tissue ADA activity was determined in nebivolol group, nebivolol treatment prevented the increase of all those enzymes activities produced by I/R. A significant decrease in spinal cord GSH-Px level was seen in I/R group.
when compared to that of control rabbits. Nebivolol treatment prevented the decrement in the spinal cord tissue GSH-Px contents produced by I/R.

I/R produced a significant increase in the spinal cord tissue MDA and NO contents, when compared with control groups. I/R-induced increments in MDA and NO levels of the spinal cord were significantly prevented by nebivolol treatment.

3.4. The biochemical analyses of oxidant stress markers in serum

I/R produced a significant increase in the XO and ADA activities in serum when compared with control group (Table 2). Nebivolol treatment significantly prevented the increase of all those enzymes activities produced by I/R. A significant decrease in SOD activities in the I/R group was determined when compared to that of control rabbits. Nebivolol treatment significantly prevented the decreases in the serum SOD activities produced by I/R. The serum SOD activities were increased to 11.70 ± 1.20 U/ml in nebivolol group (p<0.0001).

Serum MDA levels were increased in I/R group when compared with control groups. I/R-induced increments in MDA content were significantly prevented by nebivolol treatment. The serum MDA content in this group remained to be 0.24 ± 0.08 μmol/l (p<0.0001). Serum NO levels were decreased in I/R group when compared with control groups. Nebivolol treatment was caused further decrements in serum NO levels.

4. Discussion

This study demonstrates a considerable neuropotective effect of nebivolol, a potent free radical scavenger, on neurological, biochemical and histopathological status during periods of spinal cord I/R in rabbits. These findings strongly support the hypothesis that nebivolol exerts antioxidative effects. So far, cardiac effects of nebivolol have been reported in the literature. To our knowledge, this is the first report in which nebivolol was used for preventing I/R-induced oxidative stress in rabbit spinal cord.

4.1. Free radical damage in spinal cord

Free radicals and ROS have been associated with the etiology and/or progression of a number of diseases and in aging. Oxidative stress is an imbalance wherein formation of ROS overwhelms endogenous antioxidant defenses and repair capacity. Under normal conditions endogenous antioxidants including vitamins C and E and the antioxidant enzymes SOD, catalase (CAT) and GSH-Px have the capacity to scavenge the levels of ROS products. In ischemic conditions, these defense mechanisms fail to protect tissue from oxidative damage because of overproduction of oxygen radicals, inactivation of antioxidant enzymes and consumption of antioxidants in the ischemic tissue (Chan, 1996). Indeed, a decreased activity of mitochondrial SOD, GSH-Px and glutathione reductase was observed in isolated striatal mitochondria from rats subjected to ischemia and 24 h of recirculation (Sims et al., 1998). During the thoracic aorta operations, maintenance of a blood supply with removing the aortic...
Table 1
Spinal cord oxidant and antioxidant status in nebivolol, ischemia/reperfusion and control groups in rabbits

<table>
<thead>
<tr>
<th></th>
<th>MDA (μmol/g protein)</th>
<th>NO (μmol/g protein)</th>
<th>SOD (U/mg protein)</th>
<th>XO (U/g protein)</th>
<th>ADA (U/g protein)</th>
<th>MPO (ml/g protein)</th>
<th>GSH-Px (U/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n=5)</td>
<td>15.81±0.97</td>
<td>0.20±0.06</td>
<td>0.14±0.003</td>
<td>0.18±0.04</td>
<td>0.45±0.02</td>
<td>2.40±0.54</td>
<td>0.623±0.09</td>
</tr>
<tr>
<td>I/R (n=8)</td>
<td>22.21±0.88</td>
<td>1.23±0.45</td>
<td>0.43±0.03</td>
<td>0.77±0.31</td>
<td>0.74±0.01</td>
<td>4.38±0.51</td>
<td>0.514±0.04</td>
</tr>
<tr>
<td>N (n=8)</td>
<td>18.60±0.70</td>
<td>0.92±0.09</td>
<td>0.39±0.05</td>
<td>0.14±0.01</td>
<td>1.30±0.16</td>
<td>3.40±1.30</td>
<td>0.642±0.02</td>
</tr>
</tbody>
</table>

P-values
C-I/R 0.0001 0.0001 0.0001 0.0001 0.0001 0.001 0.009
C-N 0.0001 0.0001 0.0001 n.s. 0.0001 n.s. n.s.
I/R-N 0.0001 0.0001 0.0001 0.0001 0.0001 0.001 0.009

Results are expressed as mean±standard deviation. Non-parametric analyses with Mann–Whitney U-test were performed on the data.
n.s. not significant.
C: control, I/R: ischemia/reperfusion, N: nebivolol+ischemia/reperfusion.

clamp supports excess generation of ROS in spinal cord neurons, causing lipid peroxidation in cell membranes and oxidative damage to DNA and proteins. The destructive activity of ROS on membrane lipids occur at different metabolic levels, and lipids may be degraded by lipid peroxidation, a chain process known to derange the ultrastructure of neuronal membranes and inhibit the function of critical membrane-bound enzymes. Mitochondria are the primary source of cellular ATP. This ATP is generated via an ATP synthase that utilizes an inner mitochondrial membrane pH gradient as the driving force. The free hydronium ions are used to generate the pH gradient and the free electrons are passed down a series of enzyme complexes (I–IV), known as the electron transport chain (ETC), terminating at the final electron acceptor, which is molecular oxygen. This reduction of mitochondrial membrane pH gradient as the driving force. The free hydronium ions are used to generate the pH gradient and the free electrons are passed down a series of enzyme complexes (I–IV), known as the electron transport chain (ETC), terminating at the final electron acceptor, which is molecular oxygen. This reduction of molecular oxygen forms superoxide (O_2−) ions that can react with H^+ to form hydrogen peroxide (H_2O_2) and hydroxyl radicals (·OH) (Brown and Yamamoto, 2003). Therefore, a side effect of ATP generation from mitochondria is the formation of ROS, which, under physiological conditions, are “detoxified” by antioxidant systems. Mitochondria, as well as representing a source of free radicals in ischemia, are also at risk of injury from ROS with peroxidation of membrane lipids a possible key component in mitochondrial dysfunction (Bindoli, 1988). Injured mitochondria are an important source of where highly redox-reduced electron carriers in the respiratory chain transfer electrons directly to the residual O_2 during the ischemia, thereby producing superoxide (Turrens et al., 1991). On the other hand, the degradation of ATP after reperfusion by huge amount of molecular oxygen, ROS can be generated by XO. As seen our results, XO and ADA activities in both tissue and serum were found to be elevated in I/R group when compared to controls showing increased purine catabolism. Because XO generates superoxide radicals in I/R conditions, a second damage can be seen. After nebivolol administration, XO elevation was prevented.

Attempts to protect nervous tissue from an ischemic state enhance this membrane destructive activity, and the resulting outcome, known as reperfusion injury, is at present considered to be mediated by free radical forms of molecular oxygen. It is reasonable to suppose that the level of the postischemic tissue pO_2 tension may play a critical role in a massive formation of ROS. Therefore, a prime goal of neuroprotection is to reduce oxidative damage in order to salvage the spinal cord from oxidative damage.

4.2. The effects of nebivolol on biochemical variables

Rakhmatullaeva and Azimova (2003) evaluated the influence of nebivolol on lipid peroxidation processes and

Table 2
Serum oxidant and antioxidant status in nebivolol, ischemia/reperfusion and control groups in rabbits

<table>
<thead>
<tr>
<th></th>
<th>MDA (μmol/l)</th>
<th>NO (μmol/l)</th>
<th>SOD (U/ml)</th>
<th>XO (U/ml)</th>
<th>ADA (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C (n=5)</td>
<td>0.20±0.01</td>
<td>11.72±0.72</td>
<td>13.29±0.84</td>
<td>0.060±0.010</td>
<td>9.75±0.47</td>
</tr>
<tr>
<td>I/R (n=8)</td>
<td>0.28±0.02</td>
<td>10.39±0.47</td>
<td>8.99±0.02</td>
<td>0.239±0.080</td>
<td>17.24±1.58</td>
</tr>
<tr>
<td>N (n=8)</td>
<td>0.24±0.08</td>
<td>6.92±0.39</td>
<td>11.70±1.20</td>
<td>0.044±0.006</td>
<td>11.66±1.48</td>
</tr>
</tbody>
</table>

P-values
C-I/R 0.0001 0.0001 0.0001 0.0001 n.s. 0.0001
C-N 0.0001 0.0001 0.0001 n.s. 0.025 n.s.
I/R-N 0.0001 0.0001 0.0001 0.0001 n.s. n.s.

Results are expressed as mean±standard deviation. Non-parametric analyses with Mann–Whitney U-test were performed on the data.
n.s. not significant.
C: control, I/R: ischemia/reperfusion, N: nebivolol+ischemia/reperfusion.
lipid metabolism in patients with arterial hypertension. Lipid peroxidation was determined in erythrocytes, lipid levels were defined in blood from patient’s vein before and after therapy of nebivolol. They demonstrated that therapy of nebivolol suppressed the lipid peroxidation and activated antioxidant enzymes. Our study indicates that nebivolol significantly prevented the depletion of GSH-Px activities in spinal cord tissue by scavenging the free radicals produced by I/R. Also, nebivolol significantly prevented the increase of NO and MDA concentrations in the tissue of rabbit. In the present study, lipid peroxidation was monitored by measuring of MDA, which results from free radical damage to membrane components of the cells. We observed a significant increase in the MDA concentration in the spinal cord tissue of rabbits in I/R group. Nebivolol significantly attenuated the increase of MDA concentration in the tissue. This is probably due to its elimination capacity for ROS. The protection of spinal cord from IR injury by nebivolol may also due to the reduction of lipid peroxidation of neurons in spinal cord. Also, preventive effect of nebivolol in the depletion of GSH-Px activities may be due to its free radical scavenging and antioxidant activity. Our study indicated the marked elevation in NO level in the I/R group and nebivolol significantly attenuated this increment. This increment of NO generation in the spinal cord tissue of I/R group support the mechanism relating generation of NO caused by free radicals under oxidative stress. MPO is an enzyme located in leukocytes. Tissue MPO levels may suggest leukocyte infiltration into spinal cord tissue after I/R. According to our findings, MPO elevated in the tissue of I/R group and nebivolol treatment prevented this elevation protecting spinal cord. Consistently, we also observed the presence of infiltrating neutrophils and mononuclear phagocytes in the spinal cord tissue of rabbits in I/R group. Nebivolol on lipid metabolism in patients with arterial hypertension. Lipid peroxidation was determined in erythrocytes, lipid levels were defined in blood from patient’s vein before and after therapy of nebivolol. They demonstrated that therapy of nebivolol suppressed the lipid peroxidation and activated antioxidant enzymes. Our study indicates that nebivolol significantly prevented the depletion of GSH-Px activities in spinal cord tissue by scavenging the free radicals produced by I/R. Also, nebivolol significantly prevented the increase of NO and MDA concentrations in the tissue of rabbit. In the present study, lipid peroxidation was monitored by measuring of MDA, which results from free radical damage to membrane components of the cells. We observed a significant increase in the MDA concentration in the spinal cord tissue of rabbits in I/R group. Nebivolol significantly attenuated the increase of MDA concentration in the tissue. This is probably due to its elimination capacity for ROS. The protection of spinal cord from IR injury by nebivolol may also due to the reduction of lipid peroxidation of neurons in spinal cord. Also, preventive effect of nebivolol in the depletion of GSH-Px activities may be due to its free radical scavenging and antioxidant activity. Our study indicated the marked elevation in NO level in the I/R group and nebivolol significantly attenuated this increment. This increment of NO generation in the spinal cord tissue of I/R group support the mechanism relating generation of NO caused by free radicals under oxidative stress. MPO is an enzyme located in leukocytes. Tissue MPO levels may suggest leukocyte infiltration into spinal cord tissue after I/R. According to our findings, MPO elevated in the tissue of I/R group and nebivolol treatment prevented this elevation protecting spinal cord. Consistently, we also observed the presence of infiltrating neutrophils and mononuclear phagocytes in the spinal cord tissues of I/R group.

The present results suggest that nebivolol may be an extraordinary agent to protect the spinal cord from oxidative damage after thoracic aorta surgery. Further studies are warranted to define optimum dosage of this drug.

5. Conclusion

In summary, the present research demonstrate that I/R produced a significant increase in the SOD, XO, ADA and MPO activities in spinal cord tissue when compared with control group. Nebivolol treatment prevented the increase of all those enzymes activities produced by I/R. A significant decrease in spinal cord GSH-Px level was seen in I/R group and nebivolol treatment prevented the decrement in the spinal cord tissue GSH-Px contents. On the other hand, I/R produced a significant increase in the spinal cord tissue MDA and NO contents, this was prevented by nebivolol treatment. Taken together, these findings suggest that nebivolol has protective effects against neuronal damage induced by spinal cord I/R.

Acknowledgement

We are grateful to Mr. Dr. Suleyman Ozen for his skillful assistance in histological evaluation.

References

Sun, Y., Oberly, L.W., Li, Y., 1986. A simple method for clinical assay of MDA and NO contents, this was prevented by nebivolol treatment. Taken together, these findings suggest that nebivolol has protective effects against neuronal damage induced by spinal cord I/R.


