Investigation of the effect of taurine on sciatic nerve ischemia-reperfusion injury in rats

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ABSTRACT

Aim: The aim of this study was to investigate the effect of taurine on IR related changes in rat sciatic nerve tissue. Materials-Methods: A total of 32 Sprague Dawley male rats weighing between 250 and 300 grams were equally and randomly divided into four groups as follows: Controls, sham, I/R and taurine+I/R. Taurine was intraperitoneally administered at 200 mg/kg dose prior to the ischemia period in taurine+I/R group. Sciatic nerve tissues were bilaterally excised following the IR process. Tissue malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) levels were measured. Sciatic nerve tissues were histopathologically and immunohistochemically (terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end-labeling, TUNEL; inducible nitric oxide synthase, iNOS; and proliferating cell nuclear antigen PCNA) stained and examined. Results: Histopathological findings have indicated that taurine decreased the sciatic nerve damage and apoptosis. Moreover, taurine has also decreased MDA and NO levels and the number of inducible nitric oxide synthase (iNOS) positive cells, but significantly increased tissue SOD, GPx and CAT enzymatic activity levels when compared with the I/R group. Conclusion: Established data from the current study suggest that taurine treatment at 200 mg/kg dose has protective effects against I/R-mediated sciatic nerve injury.

Keywords: Sciatic nerve, ischemia reperfusion, taurine, oxidative stress, neuronal damage.
the neuronal function restoration following cerebral hypoxia (El Idrissi, 2008; Schurr and Rigor, 1987). Moreover, taurine-containing neurons in hippocampus are more resistant to ischemia-induced damage than the others (Wu et al., 1994). These reports suggest that taurine may exhibit protective effect against ischemia reperfusion (I/R) injuries. I/R-mediated tissue injury is one of the most causes of organ dysfunction. I/R process leads to ischemic fiber degeneration and oxidative injury in peripheral nerves. Schwann cells are the main target of oxidative injury depending on I/R (Iida et al., 2007). The extent of neural tissue damage is related to the ischemia duration along with cellular and biochemical interactions which occur as a result of reperfusion (Weisfeldt, 1987). It has been mentioned that taurine reduced ischemic brain injury in a dose-dependent manner (Sun et al., 2007).

If the equilibrium between oxidation and antioxidation processes is deteriorated, oxidative stress occurs as a result. During the reperfusion process, the amount of reactive oxygen species (ROS) increase, which in turn may lead to a serious destruction in a variety of molecules related to cellular functionality (Schmelzer et al., 1989). ROS synthesis causes lipid peroxidation (LPO) in cellular membranes and triggers the production of MDA, which is a prominent marker of the LPO level (Nagamatsu et al, 1996). Initiation of apoptosis via oxidative stress is an important pathogenic mechanism in I/R-related cell death (Freude et al., 2000). Nitric oxide (NO) is a signaling molecule which is produced in various cell types in neural tissue and synthesized by two isoforms of NOS: Neuronal and endothelial. iNOS, a third isoform of NOS, is expressed in many cell types as a result of inflammatory process. iNOS related NO production contributes to the inflammatory process and triggers I/R injury (Bredt and Snyder, 1994; Endoh et al., 1994). NO inhibits the mitochondrial electron transport chain and thereby increase the RNS production and peroxynitrite formation. Peroxynitrite leads to nitrosative damage in proteins, lipids and DNA, depletion in glutathione and mitochondrial enzymes (Poderoso et al., 1996). Apoptosis induced by oxidative stress is an important pathogenic mechanism in I/R-related cell death (Azad and Tomar, 2014).

In current study, proliferating cells were detected by using PCNA immunostaining. PCNA accumulates in cell nucleus during S phase of the cell cycle and it is a crucial factor for DNA repair / replication process. PCNA is commonly used as a pathophysiological marker for proliferating cells (Institute for Laboratory Animal Research, 2010). It has been reported that Schwann cells found in normal peripheral nerve reflect PCNA immunoreactivity (Ledesma et al., 2012). Moreover, it has also been mentioned that PCNA mRNA was also expressed in non-mitotic cells such as neurons (Koizumi et al., 2011). It has been demonstrated that Schwann cell proliferation and PCNA increased following sciatic nerve damage, which are important factors for peripheral nerve regeneration after damage (Sayan et al, 2004). Taurine also contributes to downregulation of pro-apoptotic proteins and upregulation of anti-apoptotic proteins (Saransaari and Oja, 2000). In the present study, we aimed to investigate whether taurine has protective effect on I/R-related sciatic nerve damage.

MATERIALS AND METHODS

Experiment Animals

The present study was approved by the Dumlupınar University Institutional Local Animal Care and Use Committee (900-35). A total of 32 Sprague-Dawley rats weighing between 250–300 grams were supplied from Dumlupınar University Experimental Research Center (Turkey) and included to the study. Animals were equally divided into four groups as follows: Controls (n:8), sham (n:8), I/R (n:8) and taurine+I/R (n: 8). Rats in sham group were only subjected to abdominal median laparotomy. Rats in I/R group were subjected to two hours of ischemia followed by three hours of reperfusion. Animals in taurine+I/R group were treated with taurine (Sigma-Aldrich Co. LLC. Taurine®, St. Louis, MO, USA), 200mg/kg, i.p prior to I/R process. Whole rats were kept in polycarbonate cages in a temperature-controlled (21±1°C) and humidity-controlled (45–55%) room, which was maintained on a 12/12 reversed light cycle. Animals were fed with a standard rat chow and allowed to drink water ad libitum.

Surgical Procedure

Rats were anesthetized with ketamine [50 mg/kg intraperitoneally (i.p.)] - romphun (20 mg/kg, i.p.) and were placed on a heating pad in order to maintain the body temperature. Ischemia was initiated via ligation of the abdominal aorta at iliolumbar artery level, which supply the hindlimb. Aorta and its branches were occluded with a non-traumatic vascular clamp (Vascu®Stop Bulldog Clamp) for 2h (18) and then clamps were removed. After then, reperfusion lasted for three hours. Incised abdomen sites were subsequently closed and animals were observed. At the end of the reperfusion period, animals were sacrificed under anesthesia and sciatic nerve tissues were bilaterally excised. Sciatic nerve tissue samples were rinsed with cold heparinized phosphate-buffered saline (PBS) to remove red blood cells or clots. Half of each sample was taken into 10% neutral buffered formalin (NBF) solution for histopathological and immunohistochemical analyses including TUNEL, inducible nitric oxide synthase (iNOS).

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and proliferating cell nuclear antigen (PCNA) antibody staining. The other part of the sciatic nerve tissue sample was placed in eppendorf tubes and were immediately stored at -80°C for biochemical analysis including measurement of tissue malondialdehyde (MDA), nitric oxide (NO), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) levels.

### Biochemical analysis

Whole tissue samples were homogenized prior to biochemical analysis via using a mechanic homogenizer (Analytik Jena speedmill plus, Germany). Malondialdehyde (MDA), nitric oxide (NO) concentrations and superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) enzymatic activity levels were measured in sciatic nerve tissue homogenate samples using commercial enzyme-linked immunosorbent assay (ELISA) kits (Cayman Inc, Ann Arbor, MI, USA) on microplate reader (BMG Labtech Spectrostar Nano, GmbH, Ortenberg, Germany).

### Histopathologic examination

Sciatic nerve tissue samples were removed and fixed in 10% NBF solution, embedded in paraffin blocks, were cut at 4 µm thickness and stained with hematoxylin and eosin (H&E). Stained slides were examined under light microscope (Olympus BX51, Tokyo, Japan) by a pathologist who was blinded to the study. Examined slides were histopathologically graded according to the edema and axonal vacuolization using a previously described scoring system (Coban et al., 2006). For each section, the vacuolization and edema were semiquantitatively graded from 0 to 3 as follows: 0-normal, 1-mild, 2-moderate and 3-severe.

### Immunohistochemical examination

Inducible nitric oxide synthase (iNOS), TUNEL and proliferating cell nuclear antigen (PCNA) were examined. Immunohistochemical stainings were performed using commercially available kits (Thermoscientific/lab Vision, Fremont, CA) and examined under light microscope (Olympus BX51, Tokyo, Japan). The number of immunopositive cells was scored as percentage. In TUNEL assay method, formalin-fixed sections were deparaffinized in xylene and rehydrated. In situ TUNEL staining was performed to detect the DNA fragmentation in apoptosis using an in situ cell death detection kit (ApopTag® Peroxidase In Situ Apoptosis Detection Kit, Millipore, Billerica, MA, USA) according to the manufacturer’s instructions and examined under light microscope (Olympus BX51, Tokyo, Japan). The number of TUNEL-positive hepatocytes per 100 hepatocytes was calculated from randomly selected fields. Apoptotic index was calculated as the percentage of apoptotic cells.

### Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.05 (GraphPad Software, Inc., California, USA). Whole data were expressed as mean ± standard error of the mean (SEM). Quantitative data were tested using the Kruskal-Wallis Analysis of Variance on Ranks and Dunn’s method was used for post-hoc testing. P values less than 0.05 were considered as statistically significant.

### RESULTS

Sciatic nerve tissue MDA, NO, SOD, GPx and CAT levels were significantly higher in I/R group, when compared with the control and sham groups (p<0.05). Tissue MDA and NO levels were prominently higher in I/R group when compared to the control and sham groups (p<0.001, Table 1), but these levels were lower in taurine+I/R group. (P < 0.05, Table 1). SOD, GPx, and CAT levels were also decreased in I/R group, when compared with the control and sham groups (p<0.001, Table 1). Tissue SDO, GPx, and CAT enzyme activities were significantly increased in taurine+I/R group, when compared with the
Table 2. Comparisons of scored histopathological and immunohistochemical values in sciatic nerve tissue samples between assay groups

<table>
<thead>
<tr>
<th>Scored Values</th>
<th>Control</th>
<th>Sham</th>
<th>I/R</th>
<th>Taurine+I/R</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sciatic nerve damage</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>2.75 ± 0.16*</td>
<td>1.00 ± 0.21†</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Apoptotic index (TUNEL)</td>
<td>2.58 ± 0.74</td>
<td>2.40 ± 0.63</td>
<td>45.13 ± 1.50*</td>
<td>8.63 ± 0.50</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>iNOS</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>33.13 ± 0.97*</td>
<td>2.38 ± 0.18</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>PCNA</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>22.75 ± 1.78*</td>
<td>23.63 ± 0.80</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>


Data are Mean±S.E.M. of eight rats for each group. *P<0.001, †P<0.05 compared to control and sham group; ‡P<0.05 compared to I/R group. Data were tested using the Kruskal-Wallis Analysis of Variance on Ranks and Dunn’s method was used for Post hoc testing. P<0.05 was considered as statistically significant. The number of TUNEL-positive cells per 100 was calculated from randomly selected fields. The apoptotic index was calculated as the percentage of apoptotic (TUNEL-positive stained) cells. The percentage of PCNA-positive and iNOS-positive cells was determined.

Figure 1. Histopathological examination of sciatic nerve tissues of assay groups, H&E x 100. Axonal cytoplasmic vacuolization, degeneration in axons of sciatic nerve, intramyelinic edema within nerve fibers in perivascular region and in endoneurial vessels were seen in the I/R group (C). Considerably preserved neuronal structures were seen in the sciatic nerve treated with Taurine (D). IE: Intramyelinic edema, VC: Vascular congestion, CV: Cytoplasmic vacuolization, AD: Axonal degeneration.
A. Control group, B. Sham group, C. I/R group, D. Taurine+I/R group

I/R group (p<0.05, Table 1). In histopathological examination, there was a prominent increment in total histopathological sciatic nerve damage score, when compared with the control and sham groups (p<0.001, Table 2). It was observed that taurine pre-treatment significantly reduced the histological scores when compared with the I/R group (p<0.05, Table 2). There was also a remarkable increase in axonal cytoplasmic vacuolization degeneration in sciatic nerve axons in the I/R group. Beside this, intramyelinic edema was observed not only in perivascular region, but also in endoneurial vessels in I/R group. However, the histological appearance of the neural tissue sections were nearly normal in taurine+I/R group (Figure 1A, B, C and D). In TUNEL method, I/R injury caused a significant increment in apoptotic index, when compared with the control and sham group (p<0.001). Although taurine administration reduced the apoptotic index, no meaningful difference was present between the I/R and taurine+I/R groups (Table 2, Figure 2A, B, C and D). I/R injury caused a prominent increase in iNOS and PCNA positive cell numbers than those of the control and sham groups (p<0.001). In addition, the number of PCNA positive cells were higher in taurine+I/R group than the controls and
Figure 2. Examination of apoptosis in sciatic nerve tissues of assay groups, TUNEL x 400. Apoptosis in the sciatic nerve tissue was examined using the TUNEL method. The number of TUNEL-positive cells per 100 was calculated from randomly selected fields. The apoptotic index was calculated as the percentage of apoptotic (TUNEL-positive stained) cells. I/R injury caused a significant increase in the apoptotic index (C). Taurine pre-treatment partially reduced the apoptotic index (D).
A. Control group, B. Sham group, C. I/R group, D. Taurine+I/R group

Figure 3. Examination of iNOS expression in sciatic nerve tissues of assay groups, iNOS x 400. iNOS expression was examined using immunohistochemical staining. The percentage of iNOS-positive cells was determined. I/R injury caused a significant increase in the number of iNOS-positive cells (C). Taurine pre-treatment partially reduced the number of iNOS positive cells (D).
A. Control group, B. Sham group, C. I/R group, D. Taurine+I/R group

sham (P < 0.01). Taurine pretreatment has partially reduced the number of iNOS-positive cells, however this reduction was not statistically significant (Table 2, Figure 3A, B, C, and D; Figure 4A, B, C and D).
Figure 4. Examination of PCNA expression in sciatic nerve tissues of assay groups, PCNA x 400. PCNA expression was examined using immunohistochemical staining. The percentage of PCNA-positive cells was determined. I/R injury caused a significant increase in the number of PCNA positive cells (C). Taurine pre-treatment increased the number of PCNA positive cells compared with sham and control group (D).
A. Control group, B. Sham group, C. I/R group, D. Taurine+I/R group

DISCUSSION

It’s a well-known fact that the oxidative nature of the I/R process may lead to numerous unsolicited outcomes and insults in living organisms and tissues. Especially during some surgical interventions of the hip, inguinal region and lower limbs, sciatic nerve could also be exposed to an ischemic state related to clamping of the reperfusing vessel branches.

The main specification of the neural tissue which differs it from other tissue types is that it is very vulnerable to anoxic conditions and may be irreversibly damaged in a relatively short time when compared with other tissues. It has been reported that the I/R process led to a significant increment in MDA and decrement in SOD levels (Sun et al., 2002). MDA is a useful quantitative marker for LPO. Güz et al have reported that taurine reduced the serum and tissue MDA levels in an experimental renal I/R model (Guz et al., 2007). In an other study by Qiao et al., it has been reported that taurine has decreased the MDA levels and increased the GPx levels (Qiao et al., 2015). In our study, we have observed that sciatic nerve I/R has led to a significant LPO accumulation when compared with the control and sham groups and besides, taurine pre-treatment has significantly reduced the LPO levels, which in turn may suggest that taurin can have protective effect against sciatic nerve I/R injury via reducing LPO. In a study by Bircan et al., it has been reported that taurine treatment decreased the nitric oxide levels in endotoxemia group (Bircan et al., 2011). Aydos et al. have reported that increase in NO levels and eNOS was prevented via taurine treatment in the I/R group (Aydos et al., 2014). In our study, the mean tissue NO level was significantly higher in I/R group than those of the control and sham groups. Our results have revealed that taurine pre-treatment has significantly reduced the sciatic nerve tissue NO levels, when compared with the I/R group. In immunohistochemical examination, taurine has reduced the number of iNOS positive cells, but this reduction was not meaningfull. Consequently, taurine may possess neuroprotective effect against I/R-mediated injury in sciatic nerve via decreasing NO levels and inhibiting iNOS activity. Anti-oxidant enzymes constitute crucial defense system which reduce detrimental effects via deactivating ROS interactions (Warner et al., 2004).

In a study by Demircioglu et al., it has been reported that taurine restored the I/R induced decrement in MDA, CuZn-SOD, GPx and CAT enzymatic activity levels (Demircioglu et al., 2011). We have observed that SOD, GPx, and CAT activity levels were decreased in I/R group, when compared with the control and sham groups. Beside this, taurine administration has significantly increased the antioxidant enzyme activity levels. Thus, we suggest that taurine may...
exert neuroprotective effect by improving the antioxidant defense system in I/R induced sciatic nerve injury condition. (Li et al., 2004), have demonstrated that taurine administration led to a prominent decrement in apoptosis rate in ischemic myocardial tissue (Li et al., 2004). In another study by Taranukhin et al., it has been reported that taurine administration significantly decreased the number of TUNEL-positive cells in ethanol-induced cellular apoptosis in cerebellar tissue (Taranukhin et al., 2010). Gu et al. have mentioned that taurine administration lessened the corpus callosum damage and decreased the neuronal cell death rate in hippocampal regions in rat brain (Gu et al., 2015). In another study by Men et al., it has been reported that apoptosis rate was decreased in lung tissues of taurine group rats, when compared with the I/R group (Men et al., 2010). In the current study, TUNEL staining has indicated that I/R mediated injury led to an increment in total number of TUNEL-positive cells. The apoptotic index was lower in taurine+I/R group, when compared with the I/R group.

Thus, our results suggest that taurine pre-treatment may improve sciatic nerve I/R injury via inhibiting the apoptotic pathways. We have found that I/R injury remarkably increased the number of PCNA positive cells, when compared with the sham group. In addition, the number of PCNA positive cells increased in taurine+I/R group and this increment was significant when compared with the control and sham groups. We suggest that although the sciatic nerve tissue was protected by taurin against I/R injury, increase in PCNA and Schwann cell proliferation may be depend upon the regeneration requirements of the nervous tissue.

CONCLUSION

Taurine administration has remarkable protective effects against I/R-mediated sciatic nerve injury. This protective effect was evident in context of decrease in MDA and NO levels, but increase in antioxidant enzyme activities including SOD, GPx and catalase in sciatic nerve tissue. As a result, we have concluded that taurine (200 mg/kg) is an effective neuroprotective agent against I/R-mediated injury in sciatic nerve, depending on its antioxidant and antiapoptotic effects.

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