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Original Article

Ischemia–Reperfusion Injury of Sciatic Nerve in Rats and Protective Role of Benidipine Hydrochloride

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ABSTRACT

Objective: Benidipine is an antihypertansive agent which elicits vasodilatation of arteries by blocking Ca2+. The aim of this study is to investigate the neuroprotective effects of benidipine on rat sciatic nerves exposed to ischemia and reperfusion (I/R).

Method: 30 male Wistar Albino rats were used in this study. The rats were divided into three groups(n=10 per group). I/R procedure was administered to the IR group, 10 μg/kg/day, i.v. benidipine was given for 2 hours (h) to the BIR group before I/R procedure. And a sham group (SG) was created. Next, histopathological and biochemical investigations were performed on sciatic nerve tissues. Superoxide dismutase (SOD), Malondialdehyde (MDA) and glutathione (GSH) were analyzed as oxidative stress markers; interleukin 1 beta (IL-1β) and tumor necrosis factor alpha (TNF-α) were analyzed as inflammatory stress markers in biochemical tests.

Results: Axonal swelling, myelin loss and disorganisation were seen in the IR group. Schwann cells showed hypertrophy, hyperplasia, blood vessels were seen as congested. But these results were under normal values and almost similiar between BIR and SG groups. In the BIR group ischemic fibere degeneration score was significiantly lower than IR group. And MDA, TNF-α, IL-1β values higher, GSH and SOD values were lower in the IR group, but in the BIR group all these values were kept within normal limits.

Conclusion: This study showed that benidipine reduced oxidative stress and inflammation in rat sciatic nerve after I/R injury.

Keywords: ischemia-reperfusion, sciatic nerve, benidipine hydrochloride

INTRODUCTION

Prolonged hypoperfusion of the peripheral nerves usually causes irreversible neuronal damage and neurologic worsening during the first 24–48 hours (h).[1,2] Some studies have mentioned that reperfusion should be achieved within 4.5 h to get rid of irreversible damage.[3,4] Therefore, blood supply to the tissues should be restored as early as possible. Unfortunately, tissue damage may be aggravated after reperfusion.[5,6] This undesirable situation makes it difficult to treat peripheral nerve ischemia. And the pathophysiological mechanism of tissue damage due to ischemia-reperfusion (I/R) injury has not been not fully elucidated. Especially, mediators secreted from endothelial cells have been reported to be effective in I/R injury.[7–10] In this present study, we used benidipine hydrochloride to reduce the damage of I/R injury in the peripheral nerves. Many previous studies mentioned that benidipine reduced the formation of lipid peroxidation and showed antioxidant activity in cells exposed to I/R damage.[11,12] Therefore, our hypothesis is that benidipine may have a protective effect in I/R damaged peripheral nerve tissues. So biochemical and histopathological effects of benidipine hydrochloride on sciatic nerve cells damaged by I/R were investigated.

METHODS

Animals

Before conducting the experiments, permission was obtained from the Animal Care and Use Committee of the Ataturk

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University, Erzurum, Turkey (11/198/25.10.2018). Thirty Wistar Albino rats (280-298 g) were kept in polypropylene cages with a 12/12 h light/dark cycle and controlled temperature (22 ± 2°C) for 1 week prior to the experiments.

Animal Groups

Three different groups (n = 10 per group) were formed.

IR group: Only I/R procedure was administered to the animals of this group.

BIR group: Benidipine hydrochloride, 10 μg/kg, iv for 2 h, was administered to the animals of this group before I/R procedure.

SG group: Sham operation was performed on this group.

Experimental Procedure

An appropriate laboratory environment was prepared for the experiments of all the animal groups. Body temperature of all of the animals was regulated at 37°C. BIR group was pretreated with benidipine hydrochloride (Deva-Turkey) (10 μg/kg/day, i.v. for 2 h). Then all of the animals were anesthetized with 25 mg/kg of thiopental sodium (Ulagay-Turkey). They were fixed to the operating table in supine position. After shaving the anterior abdominal wall of the animals, operation region was sterilized with povidone-iodine solution. Laparotomy was performed. Then, a Yasargil aneurysm clip was used to clamp the abdominal aorta. Blood flow was measured with laser-Doppler flowmetry (PF5010; Perimed Co.Ltd., Sweden). The abdominal region was closed with a sterile surgical thread and all animals were taken into separate single cages for resting. The abdominal cavity was opened from the same skin incision and the clips were removed after 3 hours. After checking the reperfusion with laser-Doppler flowmetry, the abdominal region was closed with a sterile surgical thread. After 24 hours of reperfusion time, animals were sacrificed by thiopental sodium. Animals in the SG underwent only abdominal dissection with the same methods. Left sciatic nerves of the animals were carefully removed (Figure 1). Half of the samples were taken for histopathological examination and the other half was stored in a freezer at −80°C for biochemical evaluation.

Histopathological Examination

The tissue samples were fixed in a 10% formaldehyde solution, washed under tap water, treated with alcohol, and then embedded in paraffin. Four to 5 μm sections were cut from the paraffin blocks, hematoxylin–eosin (H/E) and modified Gomori trichrome staining was administered. Ischemic fiber degeneration (IFD) values were graded according to the method specified by Mitsui et al.[13] According to their method the sections were graded as follows: grade 0: ≤2%, grade 1: 3–25%, grade 2: 26–50%, grade 3: 51–75%, and grade 4: >75%.

Figure 1. Appearance of the left sciatic nerve in a rat.

Biochemical Examination

Determination of superoxide dismutase (SOD) was performed according to the method specified by Sun et al.[14] Malondialdehyde (MDA) measurements were made according to the method specified by Ohkawa et al.[15] The absorbance of supernatant was determined at 530 nm. Total thiobarbituric acid-reactive materials were expressed as MDA. Determination of glutathione (GSH) measurements were made according to the method specified by Sedlak and Lindsay.[16] Determination of interleukin 1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) levels were performed in the same way as in the previous studies by using rat-specific sandwich enzyme-linked immunosorbent assay Rat Interleukin 1β ELISA Kit (Cat no: YHB0616Ra, Shanghai LZ) and Rat Tumor Necrosis Factor α ELISA kits (Cat no: YHB1098Ra, Shanghai LZ).[16] Analyses were performed in accordance with the manufacturers' instructions.

Statistical Analysis

The results were presented with continuous variables as mean ± standard deviation. The normality of distribution was confirmed with Shapiro–Wilk test. One-way analysis of variance was used for the comparison of three groups. As a post hoc test Tukey's HSD or Games-Howell test was used according to the homogeneity of variances. In graphical representations, the

Main Points

- Prolonged hypoperfusion of the peripheral nerves causes irreversible neuronal damage.
- I/R injury of peripheral nerves usually leads to the release of oxidative stress and acute inflammatory changes in the tissues.
- Benidipine hydrochloride showed histopathologically verified neuroprotective effects against cellular damage after I/R injury.

Figure 2. Hematoxylin (H/E) staining of the sciatic nerve tissues. (A) Hematoxylin–eosin staining of the sciatic nerve tissue obtained from the SG. Axons with normal shape and morphology and normal shape Schwann cells and blood vessels are seen. (B) H/E staining of the sciatic nerve tissue obtained from the IR group. Swollen and degenerated myelinated axon and dilated and congested blood vessel are seen. (C) H/E staining of the sciatic nerve tissue obtained from the BIR group. Decreased congestion in blood vessels and no pericellular edema are seen. ▶: myelinated axon; ▷ Schwann cell nucleus (normal shape); ⋆: blood vessel.

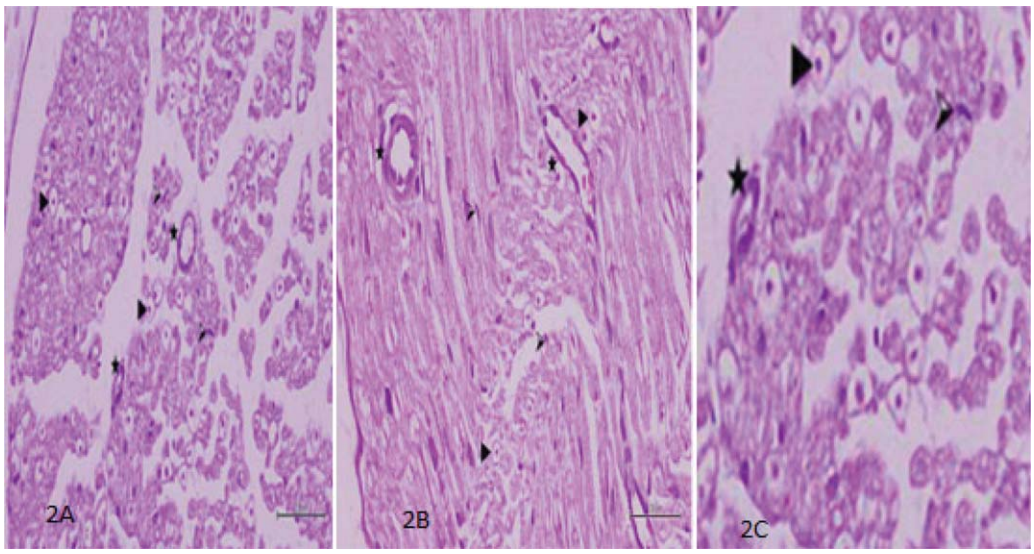
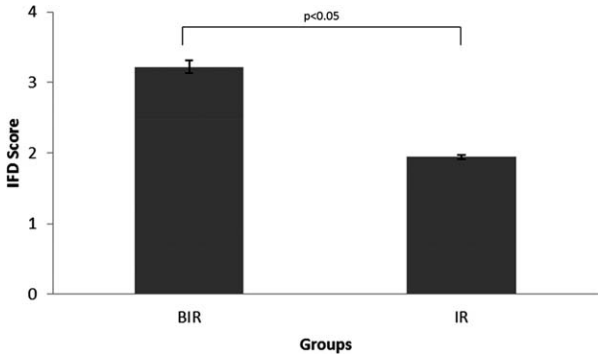


Figure 3. IFD (Ischemic fiber degeneration) scores of sciatic nerves. All data were presented as mean ± SD.



mean levels and standard errors were shown. The statistical level of significance for all tests was considered to be 0.05. Statistical Package for the Social Sciences (SPSS) Version 22.0. (IBM SPSS Corp.; Armonk, NY, USA).

RESULTS

Histopathological Findings

In the SG group, the nerve structures were generally normal, axons were located centrally and surrounded by normal myelin sheaths. Also, blood vessels were in normal shape and number. Moreover in the SG group, Schwann cells showed neither hypertrophy nor hyperplasia (Figure 2A). On the other hand, there was severe fiber degeneration, axonal swelling, and shrinkage in the IR group. The myelin loss and disorganization were found to be more in this group compared to the SG. Furthermore, Schwann cells showed hypertrophy, and hyperplasia and blood vessels were found as congested (Figure 2B). In addition, histopathological results were almost similar between BIR and SG groups. Myelinated nerve fibers were partially swollen but generally normal in sight and axons located centrally. Schwann cells were found to be normal in shape and morphology. Degeneration of myelin sheaths were decreased and blood vessels were mostly normal in the BIR group (Figure 2C).

And in the IR group, the mean IFD score was 3.22 ± 0.09, and in the BIR group, it was 1.94 ± 0.02. There was a significant difference between the IR and BIR groups about the IFD scores ($P < .05$) (Figure 3).

Biochemical Findings

SOD levels

Figure 4 shows the SOD levels in all of the groups. The mean SOD levels were found statistically lower in the IR group than SG and BIR group ($P < .05$ and $P < .05$, respectively), and there was no significant difference between SG and BIR groups ($P = .602$).

MDA levels

Figure 5 shows the MDA levels in all of the groups. Significantly ($P < .001$) higher levels of MDA were found in the IR group compared to SG and BIR groups.

Figure 4. The levels of SOD on rat sciatic nerves.

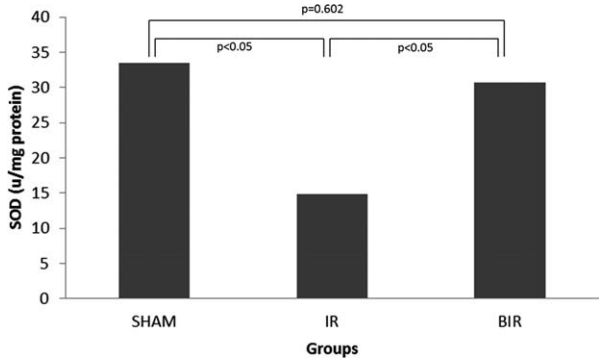


Figure 5. The levels of MDA on rat sciatic nerves.

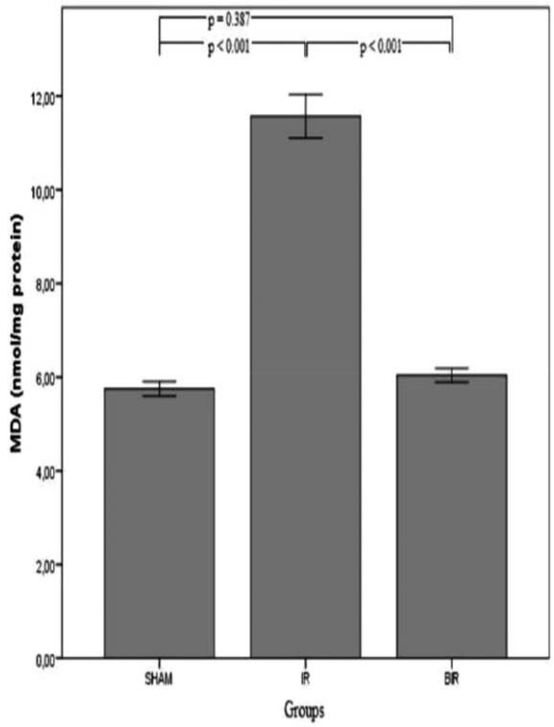


Figure 6. The levels of total GSH on rat sciatic nerves.

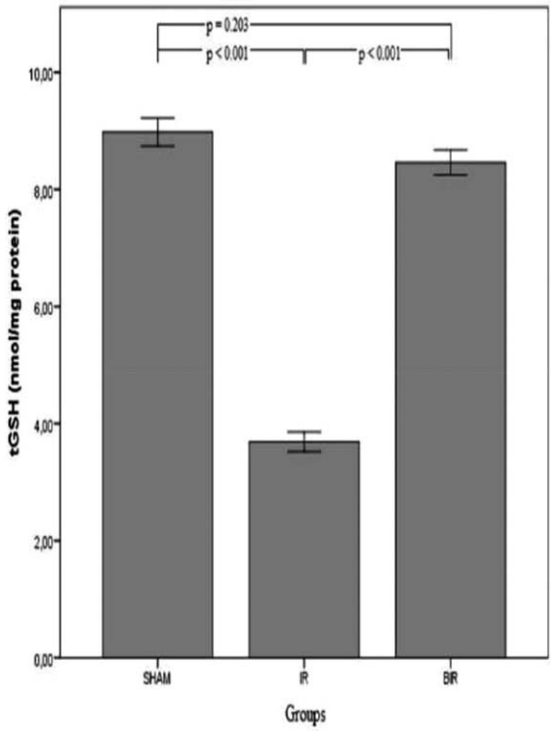
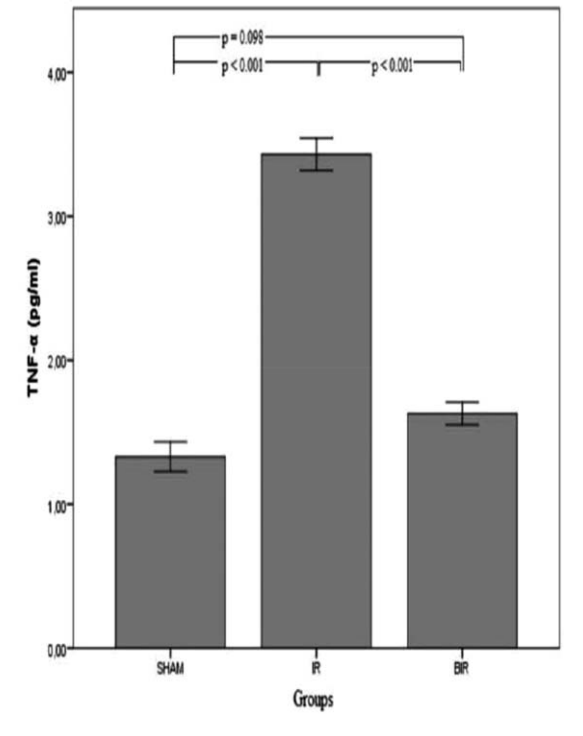


Figure 7. The levels of TNF-α levels on rat sciatic nerves.



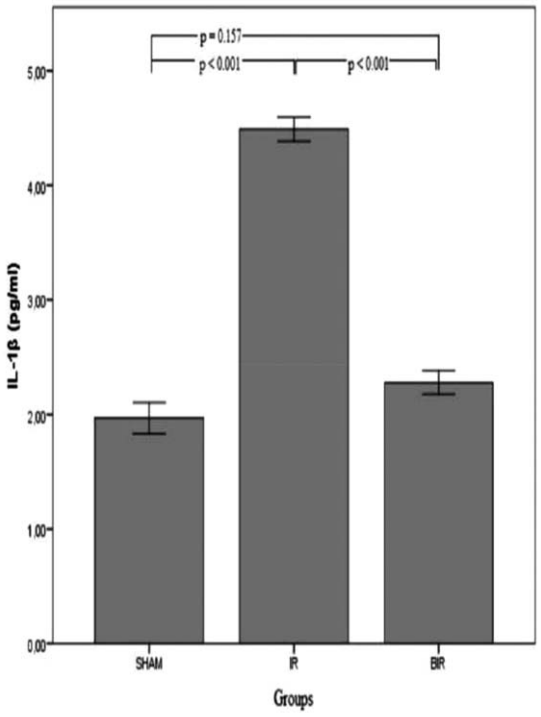
Total GSH levels

Figure 6 shows the GSH levels in all of the groups. There was a significant ($P < .001$) decrease in the levels of GSH in the IR group compared to SG and BIR groups. Pretreatment with benidipine was found to have statistically higher ($P < .001$) GSH levels in the BIR group compared to IR group.

TNF-α and IL-1β Levels

Figures 7 and 8 show the TNF-α and IL-1β levels in all of the groups. Mean TNF-α and IL-1β levels in the IR group were found to be statistically higher than that of BIR group ($P < .001$

Figure 8. The levels of IL-1β levels on rat sciatic nerves.



and $P < .001$, respectively). There were significant decreases in TNF-α and IL-1β levels in the BIR group. Furthermore, there was no significant difference between sham and BIR groups ($P = .098$, $P = .157$).

DISCUSSION

We investigated the tissue-protective effects of benidipine hydrochloride on sciatic nerve I/R injury in rats. Benidipine hydrochloride (10 μg/kg) was administered orally to the BIR group for 2 h. This dose was selected based on the previous studies, which demonstrated that administration of benidipine hydrochloride at this dose markedly reduced calcium overload in the tissues.[17,18] And benidipine showed histopathologically verified neuroprotective effects against cellular damage such as reduction of cellular swelling, hypertrophy/hyperplasia of Schwann cells, and the occurrence of myelin degeneration after I/R injury. Namely, we found significantly lower IFD levels with pretreatment of bendipine hydrochloride. Although various mechanisms have been proposed for the pathophysiology of tissue damage after I/R, the mechanism of this cascade has not been clearly known.[19,20] Many studies state that I/R injury usually leads to the release of oxidative stress and acute inflammatory changes.[2,8,21] Presumably, oxidative stress caused by I/R injury is a result of overproduction of reactive oxygen species (ROS) and excessive ROS production causes tissue damage.[22,23] Bendipine hydrochloride showed a decrease in the levels of, MDA, TNF-α, and IL-1β and an increase in GSH and SOD levels indicating a significant reduction in oxidative stress, inflammation, and an increase in antioxidant marker. According to the previous studies among calcium channel blockers, it was found that benidipine had antioxidative specifications.[11,24] Yasunari et al.[25] indicated that benidipine hydrochloride exhibited protective effect for the tissue at an approximately 100-fold lower concentration than amlodipine and nifedipine. Various methods of measuring the amount of oxidative stress in the tissues have been available in the literature.[26–28] As a sample; measurement of MDA levels has been known as one of the methods to determine the level of oxidative stress and the degree of lipid peroxidation in tissues.[29–33] It was noted by Matsubara et al.[10] that benidipine decreased lipid peroxidation and MDA levels in tissues exposed to I/R injury. And Hassan et al.[9] reported that membrane injury due to lipid peroxidation was ameliorated by the pretreatment of benidipine in myocardial cells. According to the results of our study; benidipine decreased the MDA levels and thus inhibited the formation of lipid peroxidation in sciatic nerve tissues being exposed to I/R.

Also, significantly higher GSH levels were found in the sciatic nerve tissues in the BIR group. The decrease in the antioxidative status also supports the development of oxidative stress and an increase in cellular damage.[34] Therefore, maintaining GSH, an antioxidant enzyme, in normal limits is crucial for the variety of life processes.[35] Because one of the major functions of GSH is detoxification of xenobiotics and/or their metabolites by producing oxidized glutathione in the form of glutathione disulfide. Unlubilgin et al.[16] revealed that benidipine hydrochloride prevented ovaries from the increase in oxidants and proinflammatory cytokines and they found that benidipine prevented the decrease in GSH in the tissues after I/R injury. In the light of all these findings, benidipine hydrochloride may reduce the consumption of GSH by inhibiting the increase in ROS by Ca + 2 channel blockade in tissues exposed to I/R.

It was found in our study that there were statistically significant higher levels of TNF-α and IL-1β in the IR group than BIR group. In other words, benidipine prevented the formation of acute inflammation and inflammatory injury of sciatic nerve cells exposed to I/R. As known, there has been a strong evidence that TNF-α and IL-1β are central regulators of inflammation and their antagonists have proven to be efficacious in treating inflammatory diseases.[36–39] And Yuan et al.[40] showed in their study that myocardial TNF-α and IL-1β expression were significantly less in rats treated with low-dose benidipine hydrochloride compared with untreated rats. Also, it was stated in the previous studies that the tissue protection of benidipine hydrochloride may be caused by suppression of inflammatory cytokines such as TNF-α and IL-1β.[40,41]

In addition, SOD, which is one of the markers of defense against tissue damage caused by ROS, catalyzes the dismutation of superoxide anion to hydrogen peroxide and prevents the formation of the hydroxyl radicals.[42] So SODs have been reported to alleviate inflammatory, respiratory, metabolic, cardiovascular diseases, and central nervous system disorders in the literature.[43] As a sample Ohno et al.[44] mentioned the cardiovascular protective effect of benidipine in their study and they stated that this agent increased SOD levels in the tissues. And this mechanism had been reported to be achieved by Ca + 2 channel blockade in addition to its antihypertensive efficacy.

CONCLUSIONS

Lack of neurophysiological evaluation by electromyography was the limitation of our study. On the contrary, we are at the opinion that benidipine hydrochloride may become an alternative or a supplementary agent in the treatment of axonal I/R injury.

Ethics Committee Approval: This study was approved by Ethics committee of Animal Care and Use Committee of the Ataturk University, Erzurum, Turkey (Approval No: 11/198/25.10.2018).

Informed Consent: N/A

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - T.C., S.C.Y., S.K.; Design - T.C., S.C.Y., S.K.; Supervision - T.C., S.C.Y., S.K.,H.S.; Resource - T.C., S.C.Y., G.N.Y., M.S., Y.K.A; Materials - T.C., S.C.Y.,H.S.; Data Collection and/or Processing - T.C.,G.N.Y., M.S., Y.K.A.; Analysis and/or Interpretation - T.C.; Literature Search - T.C., S.C.Y.,H.S.; Writing - T.C.; Critical Reviews - T.C., H.S.

Conflict of Interest: The authors have no conflicts of interest to declare.

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