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Laboratory Study

Effects of ceftriaxone on ischemia/reperfusion injury in rat brain

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ABSTRACT

The aim of this study was to investigate the effect of ceftriaxone treatment against short-term global brain ischemia/reperfusion (I/R) injury in rats. The study was carried out on 30 Wistar-albino rats that were divided into three groups: control group ($n = 10$), I/R group ($n = 10$) and I/R-ceftriaxone group ($n = 10$). Malondialdehyde (MDA) levels were significantly increased in the I/R group in comparison with the control group ($p < 0.001$). MDA was significantly lower in the I/R-ceftriaxone group than in the I/R group ($p < 0.05$). Superoxide dismutase activity was significantly decreased in the I/R group and increased in the I/R-ceftriaxone group as compared with the control group. Glutathione peroxidase activity was significantly decreased in the I/R group and increased in the I/R-ceftriaxone group as compared with the I/R group and the control. Histopathologically, ceftriaxone provided morphological improvement compared with the I/R group. We concluded that ceftriaxone has neuron-protective effects due to its antioxidant properties as shown by a decrease in MDA overproduction and histological improvement in brain tissue.

1. Introduction

The brain is very susceptible to energy-depriving injuries and particularly sensitive to oxygen free radical-mediated injury because it has low fuel reserves, high aerobic metabolism and low concentrations of radical scavenging enzymes.[1] There is substantial experimental evidence that free radicals are produced in the brain during ischemia and reperfusion injury.[2] These reactive species are often divided into two groups: reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as nitrous oxide (NO).

Free radicals are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidation (LPO).[3] Approaches to demonstrating their involvement in cerebral ischemic damage have concentrated on measuring the rate of consumption of endogenous protective molecules or the formation of byproducts of LPO, such as malondialdehyde (MDA).[4] Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are endogenous antioxidants which play a role in the prevention of oxidative injury.[5] An enhancement of antioxidant activities in brain tissues may be potentially beneficial for neuronal recovery from ischemia/reperfusion (I/R) injury.[6]

Beta-lactam antibiotics have lately been reported to possess neuroprotective features.[7] They have been shown to have antioxidant effects in ischemic brain injury. Ceftriaxone, a beta-lactam antibiotic, has an antibacterial activity based on the inhibition of mucopeptide synthesis in the cell wall.[8,9] Recent studies have indicated that ceftriaxone has an antioxidant role in the brain and nervous system.[10,11] However, its neuroprotective effects on global cerebral ischemia, in terms of tissue oxidant and antioxidant level and with morphological analysis, have not yet been reported in rats. Therefore the aim of the present study was to investigate the effect of ceftriaxone treatment against short-term global brain I/R injury in rats with biochemical and histological analysis.

2. Materials and methods

2.1. Animals and experimental procedures

The animals were procured, maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals by Mustafa Kemal University, Animal Ethical Committee.

Male Wistar-albino rats weighing 200–250 g were housed in polycarbonate cages and given the standard laboratory chow and water at 24 °C with 42 ± 5% relative humidity in a 12:12 hour light: dark cycle. Body temperature was maintained around 37 ± 5 °C throughout the surgical procedure.

2.2. Experimental design

Rats were divided into three groups: (i) control ($n = 10$), (ii) I/R ($n = 10$) and (iii) I/R-ceftriaxone ($n = 10$; 100 mg/kg ceftriaxone for 2 hours before I/R).

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2.3. Induction of cerebral ischemia

Rats were anesthetized with ketamine hydrochloride (75 mg/kg) and xylazine (8 mg/kg). Both common carotid arteries were exposed through lateral incisions and separated from the vagus nerve. Ischemia was induced by bilateral clamping of the common carotid arteries for 20 minutes. Reperfusion was achieved by declamping the arteries, then the circulation was restored for 20 minutes. Ceftriaxone was obtained from Roche (Basel, Switzerland) and administered intraperitoneally.

2.4. Tissue samples

Brain tissues were rapidly excised. Right brain samples were used for microscopic examination. Left brain samples were used for biochemical analyses. The brain tissue samples were stored at −70 °C until tissue analysis of MDA and NO concentration, and SOD and GSH-Px activity.

2.5. Biochemical determination

Brain tissues were weighed and homogenized (for 2 minutes at 5000 rpm) in four volumes of ice-cold Tris-HCl buffer (50 mM, pH 7.4) using a glass Teflon homogenizer (Ultra-Turrax IKA T10 Basic; IKA, Guangzhou, China) for MDA, NO and protein measurement. The homogenates were then centrifuged at 5000*g* for 60 minutes to remove debris and obtain the supernatant. Supernatant fluids were collected and and kept at −40 °C until the GSH-Px activity was measured (about 1 week later). The supernatant solutions were mixed with an equal volume of an ethanol:chloroform mixture (5:3 volume per volume [v/v]). After centrifugation at 5000*g* for 30 minutes, the clear upper layer (the ethanol phase) was collected and used in the analysis of SOD activity and protein assays. All preparation procedures were carried out at +4 °C.

2.6. Determination of malondialdehyde level

The tissue malondialdehyde (MDA) level was determined using a method from Esterbauer and Cheeseman,[12] based on its reaction with thiobarbituric acid (TBA) at 90–100 °C. In the TBA test reaction, MDA and TBA react to produce a pink pigment with an absorption maximum at 532 nm at pH 2–3 and at 90 °C for 15 minutes. The sample was mixed with two volumes of cold 10% (weight per volume [w/v]) trichloroacetic acid to precipitate the protein. The suspension was centrifuged and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling-water-bath for 10 minutes. After cooling, the absorbance was read at 532 nm. Results were expressed as nmol/g of wet tissue, by reference to a standard curve prepared from measurements made with a standard solution (1,1,3,3-tetramethoxypropane).

2.7. Determination of nitric oxide level

The method for determining plasma nitrite and nitrate levels was based on the Griess reaction. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured by spectrophotometry at 545 nm after conversion of nitrate to nitrite by copperized cadmium granules. A standard curve was established with a set of serial dilutions (10^{-8} to 10^{-3} mol/L) of sodium nitrite. Linear regression was done using the peak area from nitrite standards. The resulting equation was used to calculate the unknown sample concentrations. The NO levels were expressed as μmol/g wet tissue.

2.8. Determination of superoxide dismutase activity

Total SOD (EC 1.15.1.1) activity was determined based on the method of Sun et al.[13] The principle of the method is based on the inhibition of nitro blue tetrazolium (NBT) reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant. One unit of SOD was defined as the amount of enzyme causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as U/mg protein.

2.9. Determination of glutathione peroxidase activity

GSH-Px (EC 1.6.4.2) activity was measured. The enzyme reaction in the tube containing nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione (GSH) and sodium azide, and glutathione reductase was initiated by the addition of peroxide (H_2O_2). The change in absorbance at 340 nm was monitored with a spectrophotometer. Activity was expressed as U/g protein.

2.10. Histopathologic examination

Brain samples were fixed in 10% neutral buffered formalin for light microscopic examination. After dehydrating with a graded alcohol series, tissues were embedded in paraffin. Several 5 μm thick transverse sections were obtained from brain tissue blocks and stained with hematoxylin-eosin for histological evaluation. Sections were examined and photographed with an Olympus DP20 camera attached to an Olympus CX41 photomicroscope (Olympus, Tokyo, Japan).

2.11. Statistical analysis

Data were analyzed using SPSS 15 for Windows (SPSS, Chicago, IL, USA). Distributions of the groups were tested using the one-sample Kolmogorov-Smirnov test. The measured parameters for all groups were normally distributed, and parametric statistical method was used to analyze the data. One-way analysis of variance (ANOVA) test was performed, and post-hoc multiple comparisons were done using the least significant difference (LSD). Results were presented as mean ± standard error of the mean. A value of $p < 0.05$ was regarded as statistically significant.

3. Results

3.1. Malondialdehyde, nitrous oxide and antioxidant enzyme results

The results of measurements of MDA, NO and antioxidant enzymes are shown in Table 1. The MDA levels were significantly increased in the I/R group in comparison with the control group ($p < 0.001$). MDA was significantly lower in the I/R-ceftriaxone group than in the I/R group ($p < 0.05$). No significant differences were noted in NO levels among the groups. The mean SOD level significantly decreased in the I/R group, while mean SOD level was significantly higher in I/R-ceftriaxone group than in the I/R group. The mean GSH-Px level significantly decreased in the I/R group, while mean GSH-Px level was significantly higher in the I/R-ceftriaxone group than in the I/R group.

3.2. Histopathologic results

Microscopic examination revealed normal neuronal structure in the control group (Fig. 1). In the I/R group, shrunken neurons and eosinophilic cell bodies were observed (Fig. 2). Disruption in some

Table 1
Concentrations of malondialdehyde and nitrous oxide, and enzyme activities of superoxide dismutase and glutathione peroxidase in rats

Group	MDA (nmol/g wet tissue)	SOD (U/mg protein)	GSH-Px (U/g protein)	NO (μmol/g wet tissue)
Control (n = 10)	7.92 ± 0.47	0.279 ± 0.096	4.85 ± 0.19	0.0211 ± 0.0026
I/R (n = 10)	11.75 ± 0.30[a]	0.123 ± 0.033[a]	4.48 ± 0.28[a]	0.0251 ± 0.0037
Ceftriaxone-I/R (n = 10)	10.34 ± 0.51[a,b]	0.210 ± 0.042[a,b]	4.72 ± 0.33[a,b]	0.0245 ± 0.0029

GSH-Px = glutathione peroxidase, I/R = ischemia/reperfusion, MDA = malondialdehyde, NO = nitrous oxide, SOD = superoxide dismutase.
[a] $p < 0.001$ compared with control group.
[b] $p < 0.05$ compared with I/R group. Data are presented as mean ± standard error of mean.

microvessels (Fig. 3), meningeal congestion, hemorrhage and edema was more also observed (Fig. 4).

In the I/R-ceftriaxone group, meningeal hemorrhage persisted without any edema (Fig. 5). Microvessel structure was more preserved than in the I/R group. Neuron structure was preserved compared with the I/R group. However, shrunken neurons with eosinophilic cell bodies and pyknotic nuclei persisted in some regions (Fig. 6).

4. Discussion

According to our literature search, this study is the first report showing neuroprotective effects of ceftriaxone, with examination of tissue oxidant and antioxidant levels and morphological analysis, in a rat model of short-term global cerebral ischemia and reperfusion.

Two major hypotheses have been developed to account for the phenomenon of I/R-induced neuronal death. The neurotransmitter hypothesis is related to the role of excitotoxic amino acids and preferentially aims to explain events during the acute period of ischemia. The free radical hypothesis describes the events during reperfusion.[14] ROS lead to peroxidation of phospholipids with consecutive alteration of membrane structure. These events provide a conceptual basis for explaining the delayed neuronal death after periods of I/R.[15] Animal studies have shown that endothelial adhesion of polymorphonuclear leukocytes, which generate ROS and RNS, significantly contributes to the pathogenesis of reperfusion injury after global ischemia.[16]

The balance between the consumption of endogenous protective molecules and the formation of products of LPO, such as MDA,[4] determines tissue resistance to oxidative injury. SOD and GSH-Px are endogenous antioxidants which function in the prevention of oxidative injury.[5,6] Therefore, an enhancement in

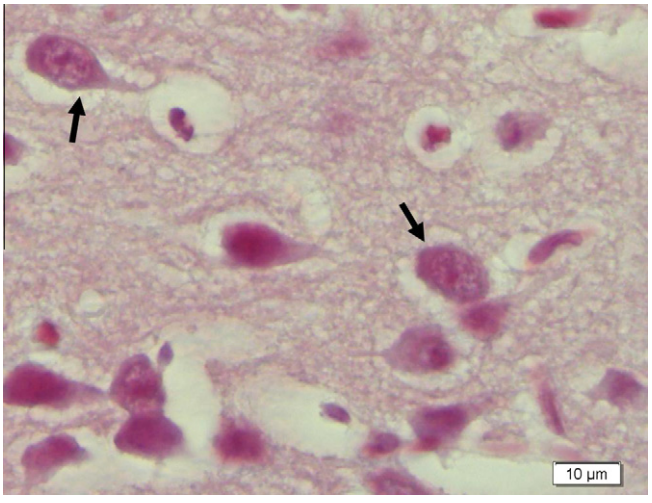

Fig. 1. Hematoxylin and eosin staining of control rat brain showing normal neurons, basophilic cytoplasm and euchromatic nuclei (arrows). (This figure is available in colour at www.sciencedirect.com.)

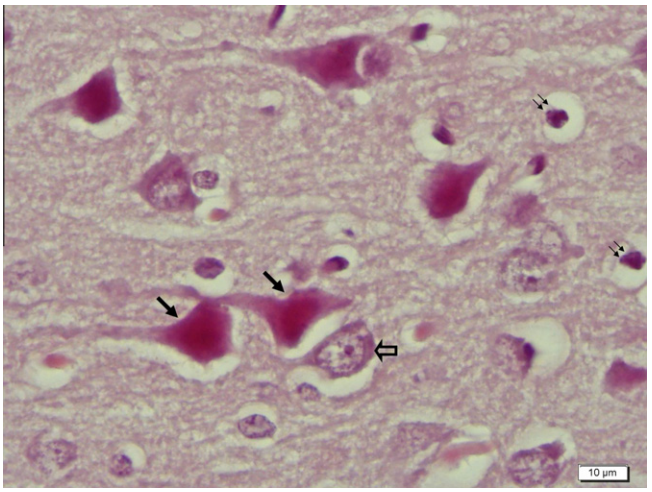

Fig. 2. Hematoxylin and eosin staining of rat brain after ischemia/reperfusion injury showing shrunken neurons (double arrows), eosinophilic cell bodies (bold arrows) and some normal neurons (open arrow). (This figure is available in colour at www.sciencedirect.com.)

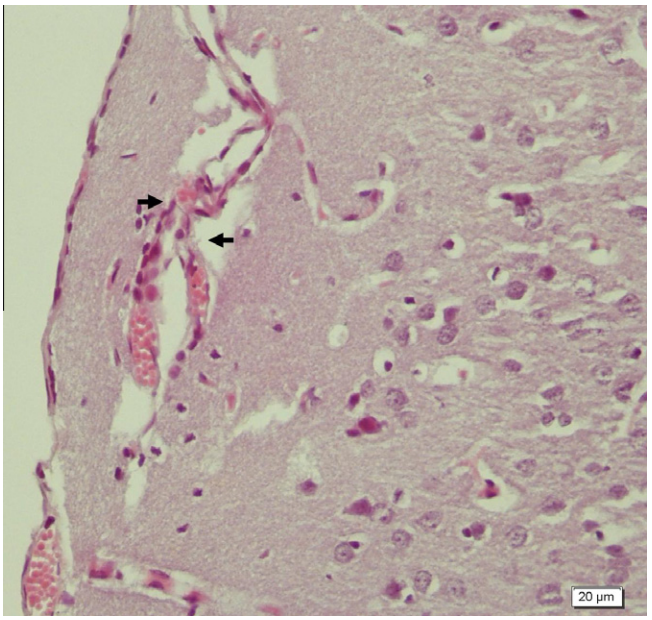

Fig. 3. Hematoxylin and eosin staining of rat brain after ischemia/reperfusion injury showing disruption in a microvessel (arrows). (This figure is available in colour at www.sciencedirect.com.)

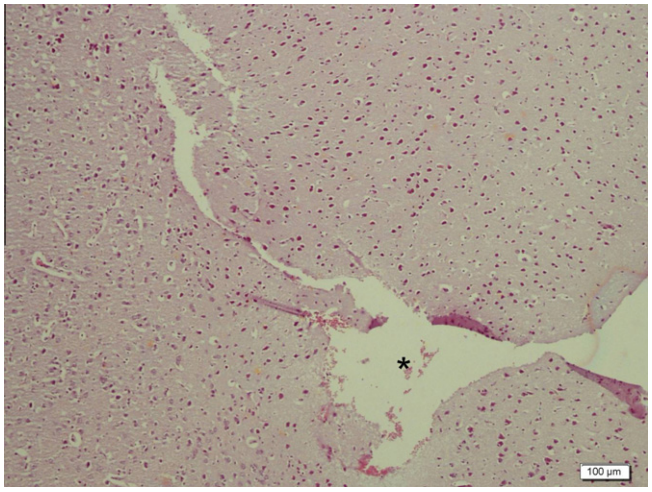

Fig. 4. Hematoxylin and eosin staining of rat brain after ischemia/reperfusion injury showing hemorrhage, meningeal congestion and edema (*). (This figure is available in colour at www.sciencedirect.com.)

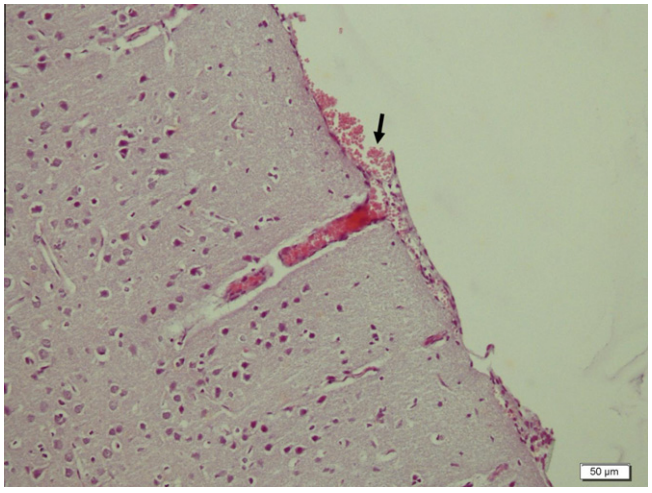

Fig. 5. Hematoxylin and eosin staining of rat brain after ischemia/reperfusion injury and ceftriaxone treatment showing meningeal hemorrhage (arrow). (This figure is available in colour at www.sciencedirect.com.)

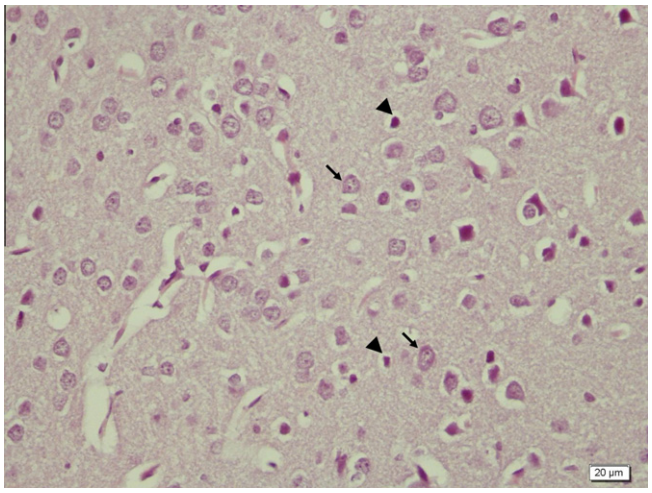

Fig. 6. Hematoxylin and eosin staining of rat brain after ischemia/reperfusion injury and ceftriaxone treatment showing shrunken neurons with pyknotic nuclei (arrowheads) and normal neurons (arrows). (This figure is available in colour at www.sciencedirect.com.)

antioxidant activity in brain tissues may be potentially beneficial for neuronal recovery from I/R injury.

Ceftriaxone has been reported to have neuroprotective effects in rodent models of amyotrophic lateral sclerosis, multiple sclerosis, stroke, neurotoxicity, Huntington's disease, depression, addiction, dependence and tolerance.[7,17] The underlying mechanism was suggested to be the increase in cellular glutamate uptake through activation of glutamate transporter subtype 1.[7]

In addition to glutamate metabolism-based neuroprotective effects, beta-lactam agents have been reported to have antioxidant effects in ischemic brain injury: Carreer et al. reported that beta-lactam agents inhibit oxidation that is mediated by hypochlorous acid (HOCL).[18] In another study by Cantin and Woods, the cytoprotective effect against HOCL could be due to the presence of the thioether group in beta-lactam agents.[19]

Recent studies have indicated that ceftriaxone has an antioxidant role in the brain and nervous system.[10,11] However, its neuroprotective effects haven't yet been reported with examination of tissue oxidant and antioxidant levels and with morphological data in a rat model of global cerebral ischemia and reperfusion. Therefore, we investigated the effect of ceftriaxone treatment against short-term global brain I/R injury in rats with biochemical and histological analysis. We found that ceftriaxone significantly decreased MDA levels and increased GSH-Px and SOD levels and improved tissue structure histologically.

MDA has been reported to increase in global cerebral ischemia models.[20] Irmak et al. showed that reperfusion injury in rat brain leads to a significant increase in MDA levels. These data are consistent with the results of the present study that ceftriaxone ameliorated LPO as shown by decreased MDA levels.

In ischemic conditions, SOD forms the principal defense system against excess O_2 production during reperfusion. The current study has also demonstrated that the erythrocyte and plasma SOD activities in rats were decreased dramatically after ischemia insult. These results are completely consistent with a previously reported study performed with antioxidant agents other than ceftriaxone.[21]

GSH, considered to be the most prevalent and important intracellular non-protein thiol, has a crucial role as a free radical scavenger. In the current study, GSH-Px content was moderately reduced due to I/R insult. Our results are compatible with other studies.[22,23] We have shown that NO levels were increased in response to I/R.

5. Conclusion

Ceftriaxone has neuroprotective effects due to its antioxidant properties, as shown by the decrease in MDA overproduction, increase in antioxidants, including SOD and GSH-Px, and histological appearance of the brain. However, the exact mechanisms underlying this beneficial effect should be clarified by further studies.

Conflicts of interest/disclosures

The authors declare that they have no financial or other conflicts of interest in relation to this research and its publication.

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