Injury, Int. J. Care Injured 46 (2015) 240–248

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Neuroprotective effects of testosterone on ischemia/reperfusion injury of the rabbit spinal cord

Bora Gürer [a,*], Hayri Kertmen [b], Emin Kasim [b], Erdal Resit Yilmaz [b], Burhan Hakan Kanat [c], Mustafa Fevzi Sargon [d], Ata Türker Arikok [e], Berrin Imge Ergüder [f], Zeki Sekerci [b]

[a] Ministry of Health, Fatih Sultan Mehmet Education and Research Hospital, Neurosurgey Clinic, Istanbul, Turkey
[b] Ministry of Health, Diskapi Yildirim Beyazit Education and Research Hospital, Neurosurgey Clinic, Ankara, Turkey
[c] Ministry of Health, Elazig Education and Research Hospital, General Surgery Clinic, Elazig, Turkey
[d] Hacettepe University, Faculty of Medicine, Department of Anatomy, Ankara, Turkey
[e] Ministry of Health, Diskapi Yildirim Beyazit Education and Research Hospital, Department of Pathology, Ankara, Turkey
[f] Ankara University, Faculty of Medicine, Department of Biochemistry, Ankara, Turkey

ARTICLE INFO

Article history:
Accepted 3 November 2014

Keywords:
Ischemia/reperfusion
Methylprednisolone
Neuroprotection
Spinal cord
Testosterone

ABSTRACT

Aim: Previous studies demonstrated the neuroprotective effects of testosterone, but no previous study has examined the neuroprotective effects of testosterone on spinal cord ischemia/reperfusion injury. The purpose of this study was to evaluate whether testosterone could protect the spinal cord from ischemia/reperfusion injury.

Methods: Rabbits were randomised into four groups of eight animals as follows: group 1 (control), group 2 (ischemia), group 3 (methylprednisolone) and group 4 (testosterone). In the control group only a laparotomy was performed. In all other groups, the spinal cord ischemia model was created by the occlusion of the aorta just caudal to the renal artery. Levels of malondialdehyde and catalase were analysed, as were the activities of caspase-3, myeloperoxidase, and xanthine oxidase. Histopathological and ultrastructural evaluations were performed. Neurological evaluation was performed with the Tarlov scoring system.

Results: After ischemia-reperfusion injury, increases were found in caspase-3 activity, myeloperoxidase activity, malondialdehyde levels, and xanthine oxidase activity. In contrast, decreases in catalase levels were observed. After the administration of testosterone, decreases were observed in caspase-3 activity, myeloperoxidase activity, malondialdehyde levels, and xanthine oxidase activity, whereas catalase levels increased. Furthermore, testosterone treatment showed improved results concerning histopathological scores, ultrastructural score and Tarlov scores.

Conclusions: Our results revealed for the first time that testosterone exhibits meaningful neuroprotective activity following ischemia-reperfusion injury of the spinal cord.

Introduction

Severe neurological injury still represents one of the most catastrophic complications after thoracoabdominal aneurysm surgery, and results from the ischemia/reperfusion (I/R) injury of the spinal cord. This devastating injury may cause paraplegia up to 40% of the patients [1]. Optimal neuroprotection is the cornerstone for success of such surgeries.

Ischemic injury consists of inadequate blood supply to the spinal cord, which is aggravated by reperfusion, and results in neuronal damage [2]. The mechanisms that underlie I/R injury are complex and multifactorial. Hypoxia with energy failure, excitoxicity, oxidative stress, inflammation, lipid peroxidation and apoptosis appear to be the most important mechanisms that cause neuronal damage after spinal cord I/R injury [2–6].

Testosterone (TES), the gonadal sex steroid hormone has various effects on numerous body tissues, including central nervous system (CNS) [7]. Testosterone, due to its lipophilic structure, can pass the blood brain barrier and influence neuronal cells [8]. Testosterone acts via androgen receptors, which are found in neurons throughout the CNS [7,9,10]. Many of the theuropathic effects of TES including those on libido, cognition and mood are mediated through the CNS [11].

* Corresponding author. Tel.: +90 506 316 42 01; fax: +90 216 575 04 06.
E-mail address: boragurer@gmail.com (B. Gürer).

http://dx.doi.org/10.1016/j.injury.2014.11.002
0020–1383/

One of the less known actions of TES is neuroprotection. Activation of androgen pathways cause neuronal differentiation, and increase in neurite overgrowth in cultured cells [12–14]. Other experiments in rodents suggest that TES causes an increase in neuronal stromal size, neuritic growth, plasticity and synaptogenesis in both motor neurons of spinal nucleus of the bulbocavernousus [15,16] and pelvic autonomic neurons [17]. Furthermore, Ogata et al. reported that TES had protective effects on spinal cord against neuronal damage induced by glutamate, and reduced the extent of spinal cord damage [18]. On the other hand, TES has shown to have antioxidant and antiapoptotic effects, which may further cause neuroprotective effects [10,19,20].

There are no previous studies that examine the neuroprotective effects of TES in spinal cord I/R injury. To complement previous neuroprotection studies, the purpose of this study was to evaluate whether TES could protect the spinal cord after I/R injury in rabbits. We also compared the TES with methylprednisolone (MP), which has been widely used for spinal cord injuries [21,22].

Materials and methods

Experimental groups

Animal care and all experiments were conducted following the European Communities Council Directive of November 24, 1986 (86/609/EEC) concerning the protection of animals for experimental use. All experimental procedures used in this investigation were reviewed and approved by the ethical committee of the Ministry of Health Ankara Education and Research Hospital Committee of Animal Ethics. Thirty-two adult male New Zealand white rabbits, weighing 2800–3500 g, were randomly divided into the following four groups (eight rabbits in each group):

Group 1: Control group ($n = 8$): laparotomy only. Rabbits underwent laminectomy, and non-ischemic spinal cord samples were obtained immediately after the surgery. No treatment was given to this group.

Group 2: Ischemia group ($n = 8$); Rabbits underwent transient global spinal cord ischemia. The rabbits received 2 cm^3 of saline (0.9% NaCl) intravenously immediately after the occlusion clamp was removed. The animals then underwent laminectomy, and spinal cord samples were removed 24 h post-ischemia.

Group 3: Methylprednisolone (MP) group ($n = 8$); Treated similarly to group 2, but the rabbits received a single intravenous 30 mg/kg dose of MP (Prednol, Mustafa Nevzat, Turkey) immediately after the occlusion clamp was removed. This dosage of the MP was selected based on earlier studies [2,23,24].

Group 4: Testosterone (TES) group ($n = 8$); treated similarly to group 2, but the rabbits received a single intraperitoneal dose of 15 mg/kg TES (Sustanon 250, Schering-Plough, Istanbul, Turkey; containing testosterone propionate 30 mg, testosterone phenylpropionate 60 mg, testosterone isocaproate 60 mg, and testosterone decanoate 100 mg) immediately after the occlusion clamp was removed. This dosage of TES was selected based on past studies [25,26]. The mixture of four TES esters with different half-life (testosterone propionate, testosterone phenylpropionate, testosterone isocaproate, testosterone decanoate) was preferred to provide more stable serum TES levels.

Anesthesia and surgical procedures

The animals were kept at an optimal (18–21 °C) room temperature, fed a standard diet and kept under a 12-h light–dark cycle. Free access to food and water was provided. The animals were anesthetised by intramuscular administration of 70 mg/kg ketamine (Ketalar, Parke Davis Eczacıbaşı, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Turkey) and were allowed to breathe freely. Body temperatures were measured using an anal thermometer (Digital Fever thermometer, Becton Dickinson, NJ, USA) and were maintained at 37 °C with a heating pad. Animals were placed in the supine position for the surgery. After sterile preparation, a 10-cm midline incision was made, and the abdominal aorta was exposed through a transperitoneal approach. Heparin (Nevparin, Mustafa Nevzat, Turkey) at a dose of 150 U/kg was administered intravenously 5 min before clamping for anticoagulation. Approximately 1 cm below the renal artery, the aorta was clamped under a surgical microscope using an aneurysm clip with 70 g of closing force (Yasargil, FE721, Aesculap, Germany). The cross clamp time was 20 min. At the end of the occlusion period, the clips were removed and restoration of blood flow was visually verified. The drugs were administered immediately after the clamp was removed. The rabbit aortic cross-clamping method, which was used in this study, is a useful method for these procedures. The 20 min ischemia period was chosen to achieve adequate injury [27]. The rabbits were allowed free access to food and water 2 h after surgery. Crede's manoeuvre was performed on animals with a neurogenic bladder at least two times a day. The animals were sacrificed 24 h after the operation by injection of 200 mg/kg pentobarbital (Nembutal, Oak Pharmaceuticals, Lake Forest, IL, USA). Spinal cord segments between L2 and L5 were carefully removed by laminectomy and used for the biochemical, histopathological and ultrastructural analyses. Blood (10 cm^3) was taken from the left ventricle for biochemical analysis. The blood samples were centrifuged at $1000 \times g$ for 5 min, and the upper clear supernatants were removed for analysis. All serum and tissue samples were stored at −80 °C until analysed. On the day of the analysis, the tissues were homogenised in physiologic saline solution and centrifuged at $1780 \times g$ for 20 min. The serum samples obtained as the upper clear supernatants of the centrifuged blood were used for the biochemical analyses.

Tissue caspase-3 activity

Caspase-3 activity was measured using an ELISA kit (Cusabio, Hubei, China), and the ELISA procedures were performed according to the manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibodies specific for caspase-3 had been pre-coated onto a microplate. Standards and samples were pipetted into the wells, and any caspase-3 present was bound by the immobilised antibody. After removal of any unbound substances, a biotin-conjugated antibody specific for caspase-3 was added to the wells. After washing, avidin-conjugated horseradish peroxidase (HRP) was added to the wells. Any unbound substances had been removed by the three washes with washing buffer. Following the washing procedure, the avidin-enzyme reagent was added to the wells. The colour develops in proportion to the amount of caspase-3 bound in the initial step. When the colour development stopped, the intensity of the colour was measured at 450 nm. Caspase-3 concentrations were calculated by comparing the absorbance values of the samples with those of standard caspase-3 solutions. The results are expressed in ng/ml.

Serum and tissue myeloperoxidase (MPO) activity

MPO activity was measured using an ELISA kit (Cusabio, Hubei, China), and the ELISA procedures were performed according to the manufacturer's instructions. This assay employs the competitive inhibition enzyme immunoassay technique. The microtitre plate provided in this kit was pre-coated with an antibody specific to MPO. Standards or samples were added to the appropriate microtitre plate wells with Biotin-conjugated MPO. A competitive inhibition reaction was initiated between the MPO (from the standards or the