Turk Neurosurg, 2023

Original Investigation

DOI: 10.5137/1019-5149.JTN.42362-22.6

Received: 15.09.2022
Accepted: 31.12.2022

Published Online: 12.05.2023

Cerebrolysin Amelioration of Spinal Cord Ischemia/Reperfusion Injury in Rabbit Model

Caghan TONGE[1], Pinar KURU BEKTASOGLU[2], Ahmet GULMEZ[1], M. Erhan TURKOGLU[1], Ata Turker ARIKOK[3], Berrin Imge ERGUDER[4], Bora GURER[5], Hayri KERTMEN[1]

[1]University of Health Sciences, Diskapi Education and Research Hospital, Department of Neurosurgery, Ankara, Turkey
[2]Sivas Numune Hospital, Department of Neurosurgery, Sivas, Turkey
[3]University of Health Sciences, Diskapi Education and Research Hospital, Department of Pathology, Ankara, Turkey
[4]Ankara University School of Medicine, Department of Biochemistry, Ankara, Turkey
[5]Istinye University Faculty of Medicine, Department of Neurosurgery, Istanbul, Turkey

Corresponding author: Pinar KURU BEKTASOGLU ✉ drpinarkuru@gmail.com

ABSTRACT

AIM: To investigate the effects of cerebrolysin on inflammation, oxidative stress, apoptosis, and neurologic recovery in the setting of an experimental rabbit model of spinal cord ischemia/reperfusion injury (SCIRI).

MATERIAL and METHODS: Rabbits were randomly divided into five groups: control, ischemia, vehicle, methylprednisolone (30 mg/kg), and cerebrolysin (5 ml/kg) group. The rabbits in the control group underwent laparotomy; the other groups underwent spinal cord ischemia and reperfusion injury for 20 min. Neurologic examination after 24 h was based on the Modified Tarlov scale. Myeloperoxidase activities, catalase and malondialdehyde levels, and caspase-3 concentrations were determined in serum and tissue samples. Serum xanthine oxidase levels were studied and histopathological and ultrastructural changes were examined.

RESULTS: After SCIRI, serum and tissue myeloperoxidase activities, malondialdehyde levels, caspase-3 concentrations, and serum xanthine oxidase activities were increased ($p<0.01–0.001$). Catalase levels were significantly diminished ($p<0.001$). Cerebrolysin treatment correlated with reduced myeloperoxidase and xanthine oxidase activities, malondialdehyde levels and caspase-3 concentrations; and with increased catalase levels ($p<0.001$, for all). The cerebrolysin group showed improved histopathological, ultrastructural, and neurological outcomes.

CONCLUSION: For the first time in the literature the current study reports anti-inflammatory, antioxidant, antiapoptotic, and neuroprotective effects of cerebrolysin in a SCIRI rabbit model.

KEYWORDS: Anti-inflammatory, Antiapoptotic, Antioxidant, Cerebrolysin, Ischemia/reperfusion, Neuroprotection

INTRODUCTION

The spine extends from the foramen magnum to the filum terminale in the spinal canal. It is an important anatomical structure in the protection and distribution of spinal cord roots, which are involved in nerve signal transmission from the brain to the body (7). The spinal cord may be damaged for reasons that include trauma, ischemia, infection, and tumor. Sensory, motor and/or autonomic dysfunctions occur in patients as a result of cord damage, depending on the neuronal function of the affected cord area. While the damage occurs primarily after injury, it is also seen due to reoxygenation and free radical formation when recirculation is introduced in a cord that has been ischemic for a time (23). This is defined as reperfusion injury or ischemia-reperfusion injury.

Caghan TONGE : 0000-0002-9921-1750
Pinar KURU BEKTASOGLU : 0000-0001-9889-9955
Ahmet GULMEZ : 0000-0001-6669-8980
M. Erhan TURKOGLU : 0000-0002-0939-3117
Ata Turker ARIKOK : 0000-0003-4256-3779
Berrin Imge ERGUDER : 0000-0002-7945-3074
Bora GURER : 0000-0003-1500-6184
Hayri KERTMEN : 0000-0002-7113-9830

Nearly 43,000–47,000 patients die every year from the thoracoabdominal aortic aneurysms (19). The 5-year survival rate is 13%–50% in aortic aneurysms treated with thoracic endovascular aneurysm repair (TEVAR) or open surgery (19). In the descending thoracic aorta, TEVAR's risk of cord tissue ischemia is 4 - 7%, which is between 2% and 28% in open surgery (18,19). Decompression after abdominal aortic surgery eliminates spinal cord ischemia, cerebrospinal fluid (CSF) drainage provides reperfusion by reducing CSF pressure, and antiedema treatments reduce spinal cord pressure (34). It has been observed that the neurological picture worsens in some patients during decompression due to spinal cord compression; this is explained by the damage caused by oxidative stress and free radicals attending spinal cord reperfusion where ischemia has been prolonged (80). After the interactions of local metabolites and oxidative stress at the membrane level on the damaged tissue and with increased vascular permeability due to ischemia, apoptotic activity may be triggered, leading to cell death (85). Rehabilitation after secondary injury is prolonged, as reperfusion ischemia significantly increases morbidity in these patients.

In this study, cerebrolysin (CER), a neurotrophic peptide mixture that mimics brain-derived neutrophic factor (BDNF); ciliary neurotrophic factor (CTNF); glial cell line-derived neutrophic factor (GDNF); and free amino acids related to the treatment protocols of dementia and vascular stroke were investigated in spinal cord ischemia and reperfusion injury (SCIRI) in an experimental rabbit model. Possible ameliorating CER effects in reperfusion damage and oxidative stress were investigated using oxidative stress, inflammation, and apoptosis markers in tissue and serum. Histopathological changes and electron microscopic ultrastructural changes were also examined (47).

MATERIAL and METHODS

Experimental Groups

Rabbits for experimental use were taken care of according to the European Communities Council Directive, September 22, 2010 (2010/63/EU). This study was approved by Saki Yenilli Laboratory Animals Facility Committee of Animal Ethics (dated 10/03/2019).

Thirty-two adult male New Zealand white rabbits were divided into the five groups randomly (weight range 2800–3750 g):

1. Control (Sham) group (n=8): Laminectomy alone was applied without any treatment.
2. Ischemia group (n=8): Transient global spinal cord ischemia was applied via 20-min clipping and clip removal.
3. Vehicle group (n=8): Transient global spinal cord ischemia was applied via 20-min clipping and clip removal. After occlusion clamp evacuation, 2 cc 0.9% NaCl was intraperitoneally given.
4. Methylprednisolone (MP) group (n=8): Rabbits received a single dose of 30 mg/kg MP (Prednol, Mustafa Nevzat, Turkey) intraperitoneally after occlusion clamp evacuation. MP dose was taken from the literature (39,58,79). MP is an anti-inflammatory and antioxidant agent that has seen long-term clinical use in spinal cord injury (42). MP maintains its value as positive control group in experimental SCIRI studies (29,30).
5. Cerebrolysin (CER) group (n=8): Intravenous injection of 5 ml/kg CER (Cerebrolysin, EVER Neuro Pharma GmbH, Austria) was given after 20-min clipping and clip removal. The CER dose was taken from the literature (50,67).

Anesthesia and Surgical Procedures

The rabbits were handled with previously described standards (40). 70 mg/kg ketamine (Ketalar, Parke Davis Eczacıbaşı, Turkey) and 5 mg/kg xylazine (Rompun, Bayer, Turkey) were applied intramuscularly. Spontaneous inhalation continued. In this experimental study, the rabbits' aortic cross-clamping model was used to create the SCIRI model. All the surgical procedures were performed as previously published (40). Ischemia was introduced with a 20-min clamping; once the clamp was removed, the subjects were reperfused for 24 h. This model is a reliable experimental model with proven efficacy that forms the basis of many SCIRI studies (29,30).

Spinal cord tissues between the L2-L5 levels were obtained. Biochemical, histopathological, and ultrastructural examinations were performed. For the biochemical examination, blood was obtained from the left ventricle. The investigations were done as previously reported from our group (39).

Serum and Tissue Caspase-3 Concentration

Enzyme-linked immunosorbent assay (ELISA) for serum and tissue caspase-3 concentration measurement was conducted according to manufacturer's guidelines (ELISA kit; Cusabio, Hubei, China). Methods for this measurement was described previously (40). The outcomes are presented in ng/mL.

Serum and Tissue Myeloperoxidase Analyses

Competitive inhibition ELISA for serum and tissue myeloperoxidase (MPO) activity was conducted (Cusabio, Hubei, China). Methods for this measurement was described previously (40). The outcomes are presented in ng/mL.

Serum and Tissue Malondialdehyde Analyses

Serum and tissue malondialdehyde (MDA) levels were measured using thiobarbituricacid (TBA) Methods for this measurement was described previously (40). The The outcomes are presented in nanomoles (nM).

Serum and Tissue Catalase Analyses

Serum and tissue catalase (CAT) levels were determined according to methods previously published in the literature (2). The outcomes are presented in IU/mL.

Serum Xanthine Oxidase Analyses

Serum xanthine oxidase (XO) activity was determined as previously described (53). Methods for this measurement was described previously (40). The outcomes are presented in mIU/ml.

Histopathological and Transmission Electron Microscopic Tissue Evaluation

Spinal cord tissues collected 24-h after SCIRI were investigated histopathologically and ultrastructurally as described previously (38,40,58,73).

Neurological Evaluation

Hindlimb neurological function was evaluated 24 h postoperatively using the modified Tarlov scoring system (30,40).

Statistical Analysis

All experiments were performed by blind investigators. Data were analyzed with GraphPad Prism 8.0 statistical software (GraphPad Software Inc., La Jolla, CA, USA). Normality was evaluated with histograms. One-way analysis of variance with post hoc Tukey's multiple comparison test was employed (comparisons between all groups) for comparison of the multiple independent groups. The data were expressed as means ± SEM. Values of $p<0.05$ were regarded as significant.

RESULTS

Serum and Tissue Caspase-3 Analyses

Among the control vs ischemia and vehicle groups, a significant difference existed in mean serum and tissue caspase-3 concentrations ($p<0.001$), and significant caspase-3 concentration increase existed in the damaged tissue after SCIRI. A significant decrease in serum and tissue caspase-3 concentration was seen in MP or CER group when compared with the ischemia group ($p<0.001$ for both). No significant difference was observed between the MP and CER groups, indicating that both treatments prevent apoptosis following SCIRI (Table I).

Serum and Tissue MPO Analyses

Among the control vs ischemia and vehicle groups, significant differences were obtained in serum and tissue MPO activities ($p<0.01$, all), SCIRI raised serum, and tissue MPO activities. When compared with the ischemia group, either MP ($p>0.05$ for serum, $p<0.001$ for tissue MPO) or CER ($p<0.001$ for serum and tissue MPO) treatment significantly decreased MPO activities. Between the MP and CER groups, no significant differences in serum and tissue MPO activities were observed (Table I). Overall, increased MPO, marker of neutrofil migration to injured tissue, showed decreased activities with CER and MP treatment.

Serum and Tissue MDA Analyses

Mean serum and tissue MDA levels ($p<0.001$) showed a significant difference among the control vs ischemia and vehicle groups, indicating that serum and tissue MDA levels increased with SCIRI. Comparing the ischemia group and the MP ($p<0.001$ for serum $p<0.01$ for tissue MDA) and CER ($p<0.001$ for serum and tissue MDA) groups showed significantly decreased MDA. Comparing the MP and CER groups showed no significant difference (Table I). According to these findings, CER and MP treatment prevented lipid peroxidation in SCIRI.

Serum and Tissue CAT Analyses

Serum and tissue CAT levels showed a significant difference among the control vs the ischemia and vehicle groups ($p<0.001$, for both), showing that serum and tissue CAT levels decreased after SCIRI. Compared with the ischemia group serum, tissue CAT levels were significantly increased in the MP and CER groups ($p<0.001$, for both). No significant differences were shown among the MP and CER groups (Table I). CAT levels decreased after SCIRI due to oxidative stress, and CER and MP treatment showed antioxidant activity via increasing CAT levels.

Serum XO Analyses

Serum XO activity was significantly increased in the ischemia

Table I: Biochemical Results by Experimental Group

Variable	Control	Ischemia	Vehicle	MP	CER	p
Serum Caspase-3 (ng/ml)	215.3 ± 31.3[a,c]	421.5 ± 55.62[a, f]	403.7 ± 54.81[c]	205.5 ± 42.2[f]	210.5 ± 34.65[h]	**<0.001**
Tissue Caspase -3 (ng/ml)	172.5 ± 53.98[a,c]	642.8 ± 153.0[a, f]	626.6 ± 116.8[c]	141.3 ± 75.44[f]	150.3 ± 63.15[h]	**<0.001**
Serum MPO (ng/ml)	2.39 ± 0.46[b, c]	5.49 ± 2.34[b]	5.65 ± 1.35[c]	3.80 ± 1.37	3.55 ± 1.33[h]	**<0.001**
Tissue MPO (ng/ml)	3.02 ± 0.78[b, c]	5.09 ± 0.96[b, f]	5.51 ± 1.46[c]	2.47 ± 1.38[f]	2.22 ± 0.77[h]	**<0.001**
Serum MDA (nmol/g tissue)	2.57 ± 0.51[a, c]	6.41 ± 1.11[a, f]	6.55 ± 1.05[c]	2.51 ± 0.57[f]	1.91 ± 1.34[h]	**<0.001**
Tissue MDA (nmol/g tissue)	3.73 ± 1.23[a, c]	10.66 ± 2.88[a, g]	10.98 ± 3.40[c]	6.39 ± 1.20[g]	5.65 ± 1.06[h]	**<0.001**
Serum CAT (IU/ml)	156.1 ± 41.97[a, c, d]	40.47 ± 11.90[a, f]	54.88 ± 11.23[c]	112.1 ± 22.62[d, f]	125.0 ± 12.99[h]	**<0.001**
Tissue CAT (IU/ml)	114.5 ± 1.79[a, c]	27.47 ± 10.80[a, f]	25.79 ± 10.92[c]	111.5 ± 12.82[f]	122.6 ± 21.0[h]	**<0.001**
Serum XO (mIU/ml)	10.13 ± 9.20[a, c]	61.25 ± 12.75[a, f]	57.50 ± 12.27[c]	6.00 ± 5.78[f]	2.75 ± 2.05[h]	**<0.001**

a: *Control vs Ischemia ($p<0.001$),* ***b:*** *Control vs Ischemia ($p<0.01$),* ***c:*** *Control vs Vehicle ($p<0.001$),* ***d:*** *Control vs MP ($p<0.01$),* ***e:*** *Control vs CER ($p<0.05$)* ***f:*** *Ischemia vs MP ($p<0.001$),* ***g:*** *Ischemia vs MP ($p<0.01$),* ***h:*** *Ischemia vs CER ($p<0.001$).* ***CAT:*** *Catalase,* ***CER:*** *Cerebrolysin,* ***MDA:*** *Malondialdehyde,* ***MP:*** *Methylprednisolone,* ***MPO:*** *Myeloperoxidase,* ***XO:*** *Xanthine oxidase.*

and vehicle groups when compared with the control group (p<0.001). Serum XO activity significantly decreased in the MP and CER groups when compared with the ischemia group (p<0.001 for both). No significant differences were shown among the the MP and CER groups. After SCIRI, increased XO indicated increased inflammatory state and the CER and MP treatment exerted anti-inflammatory activity (Table I).

Histopathological Evaluation

The light microscopy results of the control group were normal (Figure 1A). In the ischemia and vehicle groups, diffuse hemorrhage and congested areas were seen in the gray matter 24-h after SCIRI, and prominent necrosis and diffuse edema were seen in the white and gray matter. Polymorphonuclear leukocytes, plasma cells, and lymphocytes were seen to have invaded the injured areas. The ischemia group showed neuronal pyknosis, cytoplasmic eosinophilia, and loss of cytoplasmic elements (Figure 1B, C). In the MP and CER groups, spinal cord samples were protected from ischemia and reperfusion injury (Figure 1D, E). Histopathology scores of the ischemia group were higher than the control group (p<0.001, Figure 2), which were significantly lower in the MP and CER groups compared to the ischemia group (p<0.001 for both, Figure 2). No significant difference was observed among the MP and CER groups (Figure 2).

In the ischemia group, the normal motor neuron number was significantly lower than the control group (p<0.001, Figure 3). In the MP and CER groups, the normal motor neuron number was significantly higher than that of the ischemia group (p<0.001 for both, Figure 3). No significant difference was seen among the MP and CER groups. Histopathologically, CER and MP treatments prevented SCIRI (Figure 1D, E).

Ultrastructural Evaluation

In the control group, spinal cord's gray and white matter was normal ultrastructurally; neurons, membranes, intracellular organelles, nuclei, and perineuronal tissues were also normal (Figure 4 Control-A). All of the small- and medium-sized myelinated axons were normal. Only few of the large-sized myelinated axons showed mild separations in a small part of the myelin sheath (Figure 4 Control-B). This may be related with the delayed tissue fixation.

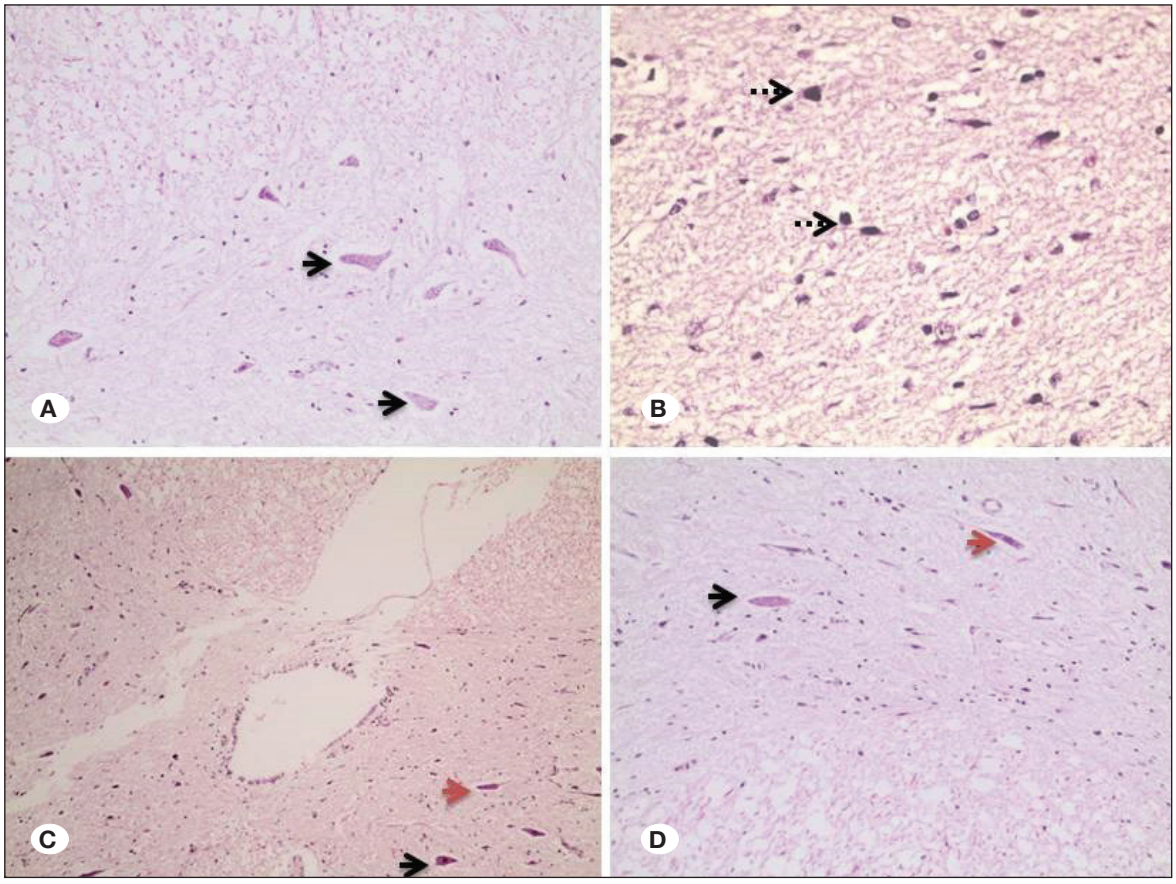

Figure 1: Photomicrographs of 5-µm spinal cord tissue sections from study groups. Images are shown with hematoxylin–eosin staining under a 40× objective. **A)** Control group, showing normal spinal cord parenchyma. Normal neurons are indicated with black arrows. **B)** Ischemia group, showing hyperchromatic degenerated neurons (dotted arrows) on the edematous surface. **C)** Methylprednisolone group, showing less degenerated neurons (orange arrows); note the normal neurons (black arrows). **D)** Cerebrolysin group, showing less degenerated neurons (orange arrows) and more normal neurons (black arrows). Spinal cord tissue was protected from injury by cerebrolysin treatment.

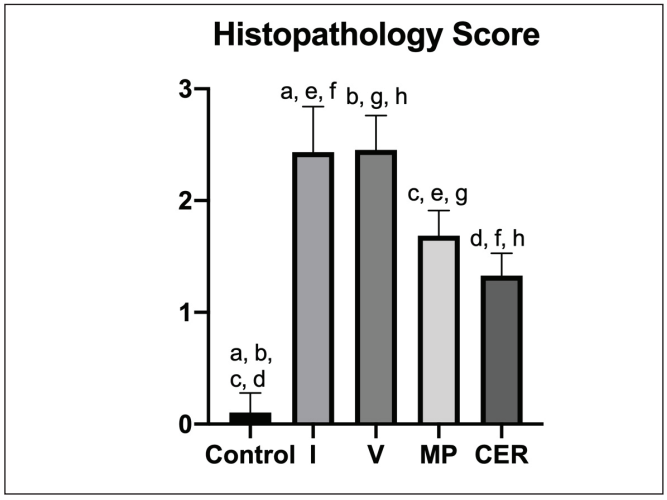

Figure 2: Distribution of histopathology score among groups. CER: Cerebrolysin, I: Ischemia, MP: methylprednisolone, V: Vehicle. a: Control vs Ischemia (p<0.001), b: Control vs Vehicle (p<0.001), c: Control vs MP (p<0.001), d: Control vs CER (p<0.001), e: Ischemia vs MP (p<0.001), f: Ischemia vs CER (p<0.001), g: Vehicle vs MP (p<0.001), h: Vehicle vs CER (p<0.001).

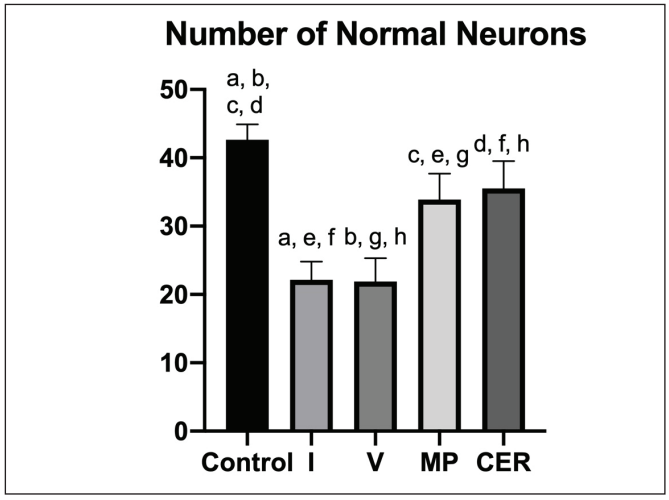

Figure 3: Distribution of normal motor neuron numbers among groups. CER: Cerebrolysin, I: Ischemia, MP: methylprednisolone, V: Vehicle. a: Control vs Ischemia (p<0.001), b: Control vs Vehicle (p<0.001), c: Control vs MP (p<0.001), d: Control vs CER (p<0.001), e: Ischemia vs MP (p<0.001), f: Ischemia vs CER (p<0.001), g: Vehicle vs MP (p<0.001), h: Vehicle vs CER (p<0.001).

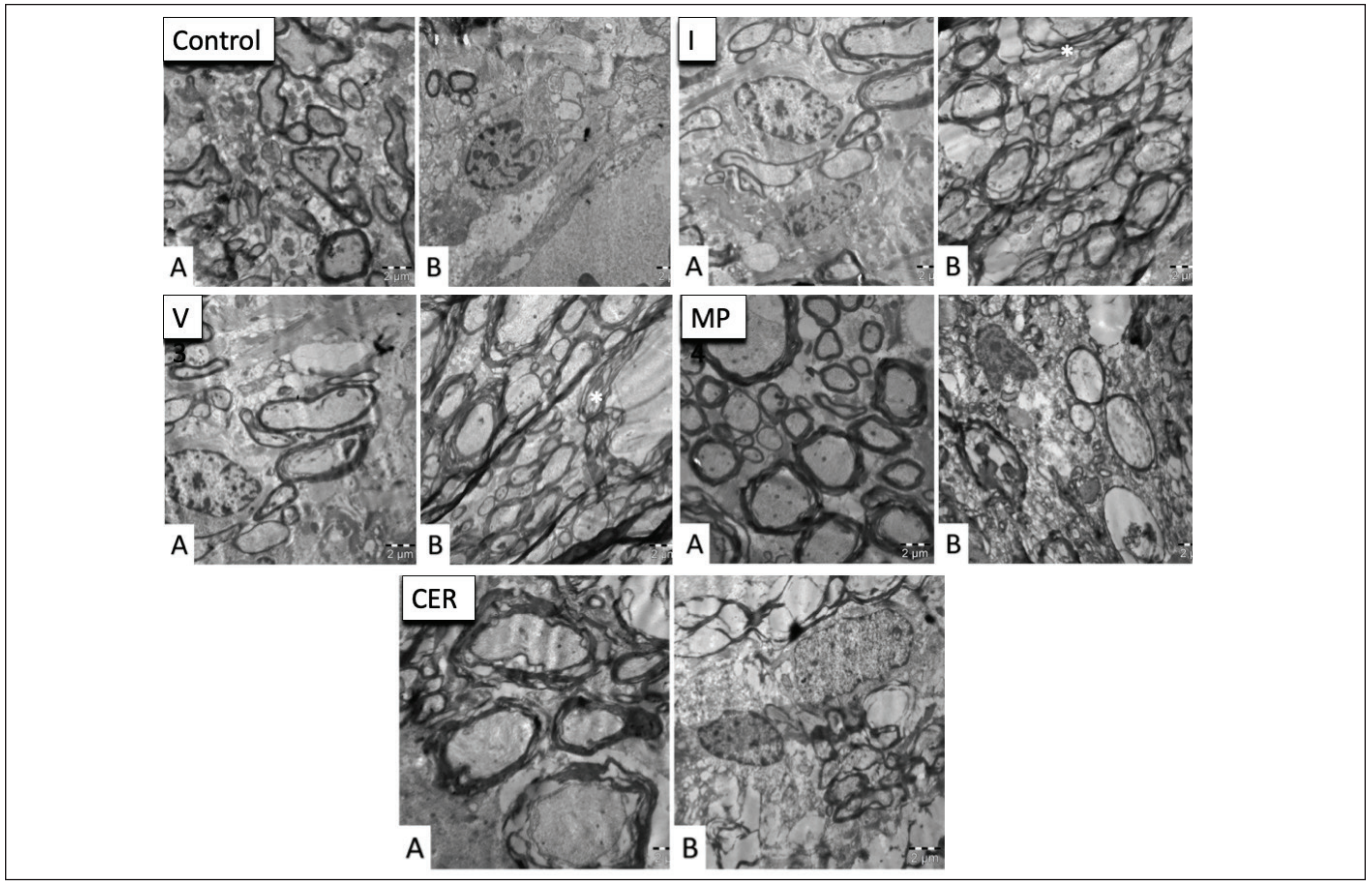

Figure 4: Each group's representative transmission electron micrographs. (Control) Control group, showing ultrastructurally normal neuronal organelles **(A)** Slight separation in large myelinated axons **(B)** (I and V) Ischemia and vehicle groups, showing intracytoplasmic vacuolization **(A)** and small, medium, and large axons with separations in myelin configuration **(B)** (MP) Methylprednisolone group, showing normal myelin configurations in small myelinated axons **(A)**, Normal intracellular structures **(B)**. (CER) Cerebrolysin group, showing small, medium, and large myelinated axons with separations in myelin configuration **(A)**, Intracytoplasmic vacuolization **(B)**. (original amplification = 5000, scale bar = 2 µm, for all). * designates axonal separation, white arrow designates vacuoles.

Table II: Electron Microscopy Results

Myelinated Axon	Control	Ischemia	Vehicle	MP	CER	p
Small-sized	0.0 ± 0.0[a, b, d]	88.40 ± 1.14[a, e]	88.40 ± 1.51[b, f]	0.0 ± 0.0[g]	90.200.0 ± 1.48[d, g]	**<0.001**
Medium-sized	0.0 ± 0.0 [a, b, c, d]	109.8 ± 1.92[a, e]	109.0 ± 2.23[b, f]	70.60 ± 2.30[c, g]	112.0 ± 1.58[d, g]	**<0.001**
Large-sized	5.0 ± 1.58 [a, b, c, d]	124.2 ± 2.04[a, e]	123.0 ± 1.0[b, f]	89.0 ± 1.58[c, g]	124.8 ± 1.30[d, g]	**<0.001**

a: *Control vs Ischemia (p<0.001),* ***b:*** *Control vs Vehicle (p<0.001),* ***c:*** *Control vs MP (p<0.001),* ***d:*** *Control vs CER (p<0.001),* ***e:*** *Ischemia vs MP (p<0.001),* ***f:*** *Vehicle vs MP (p<0.001),* ***g:*** *MP vs CER (p<0.001).* ***CER:*** *Cerebrolysin,* ***MP:*** *Methylprednisolone.*

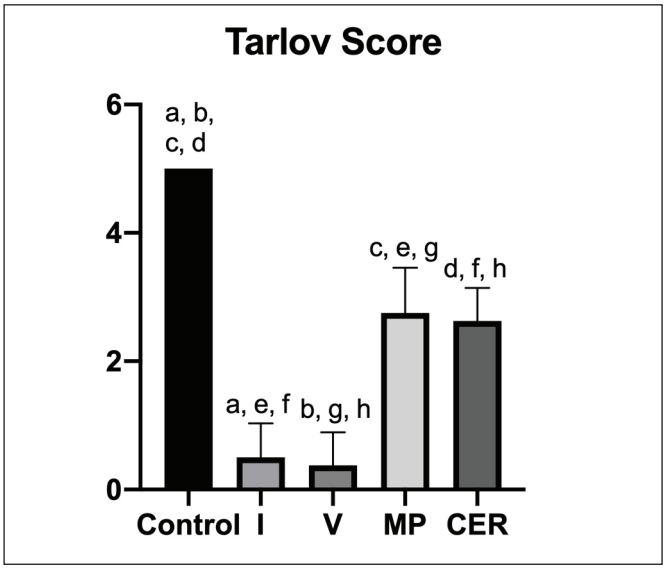

Figure 5: Distribution of Tarlov score among groups. CER = Cerebrolysin, I = Ischemia, MP= methylprednisolone, V = Vehicle. a: Control vs Ischemia (p<0.001), b: Control vs Vehicle (p<0.001), c: Control vs MP (p<0.001), d: Control vs CER (p<0.001), e: Ischemia vs MP (p<0.001), f: Ischemia vs CER (p<0.001), g: Vehicle vs MP (p<0.001), h: Vehicle vs CER (p<0.001).

In ischemia and vehicle groups, severe ultrastructural pathological changes were detected both in the gray and white matter of spinal cord tissues. Vacuoles were seen inside the neurons' cytoplasm (Figure 4, I and V-A). Perineuronal edema was also present. The nuclei and cell membranes were ultrastructurally normal in neurons. Ultrastructural pathological changes were observed in the myelinated axons (Figure 4, I and V-B). Separated myelin configurations were seen in most of the myelinated axons. In some of the medium and large myelinated axons, interrupted myelin configurations were seen. In large myelinated axons, severe ultrastructural pathological changes were observed, and these changes were lowest in small myelinated axons.

The ischemia groups had more pathological changes than the control group in all sizes of myelinated axons (p<0.001). Compared with the ischemia group, the MP treatment protected all sizes of myelinated axons from disruption (p<0.001), (Figure 4 MP-A-B). There were no difference among the ischemia and CER groups, so CER was determined ineffective in protecting myelinated axons (Figure 4 CER-A-B) (Table II).

Neurological Evaluation

In the ischemia group, the mean Tarlov score was significantly lower than the control group (p<0.001). The mean Tarlov scores in the MP and CER groups were significantly higher than those in the ischemia group (p<0.001, for both). No significant difference in the Tarlov scores among the MP and CER groups was observed (Figure 5).

DISCUSSION

During thoracoabdominal surgery, transient spinal cord ischemia and spinal cord injury is observed due to aortic cross-clambing (14,69). A large-scale study reported the spinal cord injury rate as 16%, and approximately half of these cases remained paraplegic (69). Cellular glycogen and energy stores are rapidly depleted as the blood flow to the neuronal tissue decreases, and ischemic pathways that will cause cellular death are activated (29). Reperfusion after ischemia increases cellular death due to increased apoptotic activity, oxidative stress and inflammation (29,30,39). Neuronal damage in spinal cord ischemia mainly occurs by a sequence of two mechanisms. Primary damage, blood flow cessation to the spinal cord resulting in decreased oxygen supply to the tissue, is irreversible. However, secondary damage, the activation of metabolic cascades triggered by primary damage, may be reversible (20). To prevent and treat SCIRI, CSF drainage (56), hypothermia (15,36), and shunting have been investigated (11) and several pharmacological agents have been experimentally studied to treat this damage (29,30,39).

Cerebrolysin is a hydrolyzed cerebroprotein complex containing free amino acids and neurotrophic substances (43). It is 85% free amino acids and 15% biologically active small purified neuropeptides (26). Therefore, it contains many neurotropic factors and free amino acids (48). CER has been used in neurological disease treatments for more than half a century (9,25,28,83,84). Most of its neuroprotective activity is due to its similarity to endogenous neurotrophic factors. CER neuropeptides cross the blood-brain barrier and penetrate the neuroglial tissue after intravenous injection, in contrast to natural endogenous neurotrophic factors (BDNF, CTNF, GDNF) (45,83). As a neuroprotective agent, CER increases neuronal survival, neuroplasticity, and neurogenesis (32,83,84). Animal experiments on ischemic brain tissue have shown that it reduces ischemia volume, prevents brain edema, and provides significant protection by increasing neuronal survival (27,54,83,84). Defined as an effective treatment in ischemic stroke patients, CER treatment has been used clinically (9,81,82). It has also

been applied in hemorrhagic stroke patients besides ischemic stroke (6,51). Similarly, CER applied in traumatic brain injury patients yielded good outcomes (41,76).

Neurotrophic factors such as BDNF and GDNF have proven neuroprotective efficacy after SCIRI (13,71). NGF and CNTF are other molecules with neurotrophic properties in the central nervous system (61-67). Following cytokine release after tissue damage, neurotrophic growth factors such as BDNF, GDNF, NGF, and CNTF are upregulated and increased within the tissue to provide neuroprotection; however, endogenous increases alone cannot reach levels that will protect the spinal cord from degeneration (61-67). To provide neuroprotection, these neurotrophic factors must be combined and given in high doses (61-67). CER chromatography revealed 17 different amino acids and BDNF, NGF, GDNF, CNTF, and insulin-like growth factor-1 and 2 (48). In traumatic spinal cord injury, CER has shown a significant neuroprotective effect through these neurotrophic factors (4).

Apoptosis is the most important cellular death pathway in multicellular mammalian tissues (17). An important indicator of DNA fragmentation, caspase-3 levels increase in the tissue and serum following ischemic spinal cord injury (57). Extracellular signal-regulated kinase (ERK) mediates axonal growth and neuron survival (37,72). In cortical, hippocampal, and motor neurons, ERK 1/2 is activated by BDNF (33,46). ERK inhibition causes caspase-3 activation and accelerates apoptosis in spinal cord ischemia (13). Therefore, BDNF infusion is considered to be antiapoptotic (78). Studies have shown that CER is antiapoptotic (8,21) and, following cerebral infarction, shows antiapoptotic properties via Bcl-2/Bax systems (77). Apoptosis is one of the important pathway for neuronal loss in SCIRI (60). It has been shown that the caspase-3 mechanism's reactivity increases after 15 minutes of ischemia in the spinal cord (57). Many SCIRI studies have used caspase-3 activation as a reliable marker of apoptosis (29,30). This study saw an increase in both serum and tissue caspase-3 activity in ischemia and vehicle groups following SCIRI, and caspase-3 activation was considered an active apoptosis manifestation. Following CER and MP treatments, serum and tissue caspase activities were significantly decreased. From these findings we can conclude that at a statistically significant level, CER prevents apoptosis after SCIRI.

SCIRI mrkedly increases reactive oxygen radicals in the tissue, forming one of the leading causes of neuronal causes via lipid peroxidation, membrane protein destruction, and DNA damage (12). CER's binding to free oxygen radicals and antioxidant properties have been reported in the literature (1). CAT, superoxide dismutase (SOD), and glutathione peroxidase (55) are the most important endogenous antioxidant mechanisms. CER activates free radical binding systems by increasing CAT and SOD activities, preventing mitochondrial damage in nerve cells (31). In rats with brain damage, CER increases SOD levels (24). CAT reduces oxidative stress by binding oxygen free radicals (35). CAT activity diminishes in cases of increased oxidative stress, such as SCIRI (74). In this study and as in many previous SCIRI studies, both serum and tissue CAT levels decreased due to oxidative stress in ischemia and vehicle groups. In both the serum and tissue, CER and MP treatments increased CAT levels. Thus, CAT levels decrease secondary to increased oxidative stress in the tissue due to SCIRI, while CER treatment increases the CAT level via antioxidant activity.

The XO enzyme is another marker of increased oxidative stress after SCIRI (29,30). In this study, serum XO levels increased due to oxidative stress in ischemia and vehicle groups following SCIRI. Compared to the ischemia and vehicle groups, XO levels were significantly decreased in the CER and MP groups. Its reduction of serum XO levels is another indicator of CER's antioxidant activity.

Lipid peroxidation occurs as a result of excessive production of free oxygen radicals and causes the fragmentation of vital components of membrane lipids and deterioration of membrane permeability (22). Lipid peroxidation decreases in increased stress conditions like ischemia, acidosis, and bacterial toxins (3,10,44). Due to its lipid involvement, lipid peroxidation after ischemia and reperfusion injury is a principal mechanism of cellular death (59). MDA, the end product of lipid peroxidation, is an accepted marker for peroxidation reactions (75). In severe tissue injury, cross-linkage of MDA with membrane lipids is increased (16). CER has been shown to reduce MDA level induced by subcutaneous insulin therapy in the lipid peroxidation model (52). In myocardium (5) and cerebellum tissue (68), CER has reduced lipid peroxidation. Following SCIRI, serum and/or tissue MDA levels increases (29,30). This study found serum and tissue MDA levels increased in ischemia and vehicle groups when compared to control group as a lipid peroxidation marker following ischemia and reperfusion injury. CER and MP treatments significantly inhibited lipid peroxidation and decreased both serum and tissue MDA levels. In the light of these data, it was concluded that CER treatment reduced lipid peroxidation and cell membrane degradation resulting from SCIRI.

MPO activity shows neutrophil infiltration of damaged areas (58,70) and increased MPO activity is a marker for inflammatory activity and severity (70). In SCIRI models, serum and/or tissue MPO levels are preferred as an inflammatory response indicator (29,30). Many studies have shown CER's ability to reduce neuroinflammation (4,27,49). Similar to previous studies, this study found serum and tissue MPO activity to increase in ischemia and vehicle groups after SCIRI. High MPO activity, an indicator of increased inflammation, was astatistically significantly reduced with CER and MP treatments; as a result, we concluded that both drugs were anti-inflammatory.

Among the study's ischemia and vehicle groups, light microscopy revealed significant hemorrhagic areas, congestion, edema, necrosis, and inflammation due to SCIRI in the tissues included. Significantly degenerated neuron numbers were increased in both groups, while normal morphological neuron numbers were decreased. Polymorphonuclear leukocytes, lymphocytes and monocytes infiltrated the damaged tissue, indicating neuroinflammation. It was observed that tissue morphology was better preserved in the tissues of the CER and MP groups compared to the ischemia and vehicle groups, seemingly due to the anti-inflammatory antiapoptotic, and antioxidant effects of both drugs. The number of neurons

with normal morphology was higher in both the CER and MP groups compared to the ischemia and vehicle groups.

To examine the myelin sheaths in greater detail, all groups were scanned with transmission electron microscopy. In the ischemia and vehicle groups, clear dissociation was observed in myelinated axons of all sizes. MP treatment caused improvement in myelin detachments of all sizes; no improvement in ultrastructural structure was observed in the CER group.

Neurological examination showed all subjects in the ischemia and vehicle groups to be paraplegic secondary to significant neurological damage. In the CER and MP groups, biochemical and histopathological improvement were observed and the spinal cord was protected functionally. Compared to the ischemia and vehicle groups, the modified Tarlov scores of the subjects belonging to the CER and MP groups were higher.

Given these findings, we conclude that CER has neuroprotective properties, showing antiapoptotic, anti-inflammatory, and antioxidant activity on SCIRI. There are limitations to this study. A greater number of subjects and dose-dependent investigations with different dose ranges and amounts would have strengthened the study. Drug efficacy can be studied in long-term by extending the reperfusion time. Finally, possible neuroprotective mechanisms of CER should be examined in more detail with other biochemical parameters.

CONCLUSION

This study, for the first time in the literature, reports that CER has antiapoptotic, antioxidant, and anti-inflammatory properties on SCIRI and is significantly neuroprotective. We believe that CER, long used in humans and shown many times to be safe, can be used to prevent and treat SCIRI.

ACKNOWLEDGMENTS

We would like to thank Prof. Mustafa Fevzi Sargon for his scientific contribution for investigating the histopathological specimens. Preparation for publication of this article is partly supported by Turkish Neurosurgical Society. The authors would like to thank Enago (www.enago.com) for the English language review.

AUTHORSHIP CONTRIBUTION

Study conception and design: CT, PKB, BG, HK
Data collection: CT, BG, AG, ATA, BIE
Analysis and interpretation of results: PKB, ATA, BIE
Draft manuscript preparation: CT, PKB
Critical revision of the article: MET, BG, HK
Other (study supervision, fundings, materials, etc...): MET
All authors (CT, PKB, AG, MET, ATA, BIE, BG, HK) reviewed the results and approved the final version of the manuscript.

REFERENCES

1. Abdel-Salam OME, Omara EA, Mohammed NA, Youness ER, Khadrawy YA, Sleem AA: Cerebrolysin attenuates cerebral and hepatic injury due to lipopolysaccaride in rats. Drug Discov Ther 7(6):361-371, 2013
2. Aebi H: Catalase in vitro. Methods Enzymol 105:121-126, 1984
3. Agardh CD, Zhang HZ, Smith ML, Siesjö BK: Free radical production and sichemic brain damage: Influence of postischemic oxygen tension. Int J Dev Neurosci 9(2):127-138, 1992
4. Alvarez XA, Lombardi VR, Fernández-Novoa L, García M, Sampedro C, Cagiao A, Cacabelos R, Windisch M: Cerebrolysin reduces microglial activation in vivo and in vitro: A potential mechanism of neuroprotection. J Neural Transm Suppl 59:281-292, 2000
5. Ardjmand A, Shahaboddin ME, Mazoochi T, Ghavipanjeh G: Ameliorative effects of cerebrolysin against isoproterenol-induced myocardial injury in male rats. Life Sci 227:187-192, 2019
6. Bajenaru O, Tiu C, Moessler H, Antochi F, Muresanu D, Popescu BO, Novak P: Efficacy and safety of Cerebrolysin in patients with hemorrhagic stroke. J Med Life 3(2):137-143, 2010
7. Bogduk N: Functional anatomy of the spine. Handb Clin Neurol 136:675-688, 2016
8. Boshra V, Atwa A: Effect of CER on oxidative stress-induced apoptosis in an experimental rat model of myocardial ischemia. Physiol Int 103(3):310-320, 2016
9. Brainin M: Cerebrolysin: A multi-target drug for recovery after stroke. Expert Rev Neurother 18(8):681-687, 2018
10. Bralet J, Bouvier C, Schreiber L, Boquillon M: Effect of acidosis on lipid peroxidation in brain slices. Brain Res 539(1):175-177, 1991
11. Brodbelt AR, Stoodley MA: Post-traumatic syringomyelia: A review. J Clin Neurosci 10(4):401-408, 2003
12. Chan PH: Role of oxidants in ischemic brain damage. Stroke 27(6):1124-1129, 1996
13. Chou AK, Yang LC, Wu PC, Wong WT, Liu GS, Chen JT, Howng SL, Tai MH: Intrathecal gene delivery of glial cell line-derived neurotrophic factor ameliorated paraplegia in rats after spinal ischemia. Brain Res Mol Brain Res 133(2):198-207, 2005
14. Crawford ES, Crawford JL, Safi HJ, Coselli JS, Hess KR, Brooks B, Norton HJ, Glaeser DH: Thoracoabdominal aortic aneurysms: Preoperative and intraoperative factors determining immediate and long-term results of operations in 605 patients. J Vasc Surg 3(3):389-404, 1986
15. Dietrich WD, Levi AD, Wang M, Green BA: Hypothermic treatment for acute spinal cord injury. Neurotherapeutics 8(2):229-239, 2011
16. Dröge W: Free radicals in the physiological control of cell function. Physiol Rev 82(1):47-95, 2002
17. Elmore S: Apoptosis: A review of programmed cell death. Toxicol Pathol 35(4):495-516, 2007

18. Etz CD, Kari FA, Mueller CS, Silovitz D, Brenner RM, Lin HM, Griepp RB: The collateral network concept: A reassessment of the anatomy of spinal cord perfusion. J Horac Cardiovasc Surg 141(4):1020-1028, 2011
19. Etz CD, Weigang E, Hartert M, Lonn L, Mestres CA, Di Bartolomeo R, Bachet JE, Carrel TP, Grabenwöger M, Schepens MA, Czerny M: Contemporary spinal cord protection during thoracic and thoracoabdominal aortic surgery and endovascular aortic repair: A position paper of the vascular domain of the European Association for Cardio-Thoracic Surgery. Eur J Cardiothorac Surg 47(6):943-957, 2015
20. Fan L, Wang K, Dhi Z, Die J, Wang C, Dang X: Tetramethylpyrazine protects spinal cord and reduces inflammation in a rat model of spinal cord ischemia-reperfusion injury. J Vasc Surg 54(1):192-200, 2011
21. Formichi P, Radi E, Battisti C, Maio G, Muresanu D, Federico A: Cerebrolysin administration reduces oxidative stress-induced apoptosis in lymphocytes from healthy individuals. J Cell Mol Med 16(11):2840-2843, 2012
22. Fridovich I: The biology of oxygen radicals. Science 201(4359):875-880, 1978
23. Ghincea CV, Ikeno Y, Aftab M, Reece TB: Spinal cord protection for thoracic aortic surgery: Bench to bedside. Semin Thorac Cardiovasc Surg 31(4):713-720, 2019
24. Gonzalez ME, Francis L, Castellano O: Antioxydant systemic effect of short-term cerebrolysin administration. J Neural Transm Suppl 53:333-341, 1998
25. Gschanes A, Eggenreich U, Windisch M, Crailsheim K: Early postnatal stimulation influences passive avoidance behaviour of adult rats. Behav Brain Res 93(1-2):91-98, 1998
26. Gschanes A, Valouskova V, Windisch M: Ameliorative influence of a nootropic drug on motor activity of rats after bilateral carotid artery occlusion. J Neural Transm (Vienna) 104(11-12):1319-1327, 1997
27. Guan X, Wang Y, Kai G, Zhao S, Huang T, Li Y, Xu Y, Zhang L, Pang T: Cerebrolysin ameliorates focal cerebral ischemia injury through neuroinflammatory inhibition via CREB/PGC-1α pathway. Front Pharmacol 10:1245, 2019
28. Gutmann B, Paier BH, Skofitsch G, Windisch M, Gmeinbauer R: In vitro models of brain ischemia: The peptidergic drug cerebrolysin protects cultured chick cortical neurons from cell death. Neurotox Res 4(1):59-65, 2002
29. Gurer B, Karakoc A, Bektasoglu PK, Kertmen H, Kanat MA, Arikok AT, Erguder BI, Sargon MF, Ozturk OC, Celikoglu E: Comparative effects of vitamin D and methylprednisolone against ischemia/reperfusion injury of rabbit spinal cords. Eur J Pharmacol 813:50-60, 2017
30. Gurer B, Kertmen H, Kasim E, Yilmaz ER, Kanat BH, Sargon MF, Arikok AT, Erguder BI, Sekerci Z: Neuroprotective effects of testosterone on ischemia/reperfusion injury of the rabbit spinal cord. Injury 46(2):240-248, 2015
31. Han ZT, Liu J, Zhang BL, Zhang JL, Li WB, Tao GS: Experimental study of influence on SOD and MDA of mice on the treatment of cerebrolysin. Zhongguo Laonian Xue Zazhi 24:447-448, 2004
32. Heiss WD, Brainin M, Bornstein NM, Tuomilehto J, Hong Z, Cerebrolysin Acute Stroke Treatment in Asia (CASTA) Investigators: Cerebrolysin in patients with acute ischemic stroke in Asia: Results of a double-blind, placebo-controlled randomizel trial. Stroke 43(3):630-636, 2012
33. Hetman M, Kanning K, Cavanaugh JE, Xia Z: Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. J Biol Chem 274(32):22569-22580, 1999
34. Hu J, Yu Q, Xie L, Zhu H: Targeying the blood-spinal cord barrier: A therapeutic approach to spinal cord protection against ischemia-reperfusion injury. Life Sci 158:1-6, 2016
35. Ilhan A, Yilmaz HR, Armutcu F, Gurel A, Akyol O: The protective effect of nebivolol on ischemia/reperfusion injury in rabbit spinal cord. Prog Neuropsychopharmacol Biol Psychiatry 28(7):1153-1160, 2004
36. Kakinohana M, Taira Y, Marsala M: The effect of graded postischemic spinal cord hypothermia on neurological outcome and histopathology after transient spinal ischemia in rat. Anesthesiology 90(3):789-798, 1999
37. Kaplan RD, Miller FD: Neurotropin signal transduction in the nervous system. Curr Opin Neurobiol 10(3):381-391, 2000
38. Kaptanoglu E, Palaoglu S, Surucu HS, Hayran M, Beskonakli E: Ultrastructural scoring of graded acute spinal cord injury in the rat. J Neurosurg 97 Suppl 1:49-56, 2002
39. Kertmen H, Celikoglu E, Ozturk OC, Gurer B, Bozkurt H, Kanat MA, Arikok AT, Erguder BI, Sargon MF, Sekerci Z: Comparative effects of methylprednisolone and tetracosactide (ACTH1-24) on ischemia/reperfusion injury of the rabbit spinal cord. Arch Med Sci 14(6):1459-1470, 2018
40. Kertmen H, Gurer B, Yilmaz ER, Sanli AM, Sorar M, Arikok AT, Sargon MF, Kanat MA, Erguder BI, Sekerci Z: The protective effect of low-dose methotrexate on ischemia-reperfusion injury of the rabbit spinal cord. Eur J Pharmacol 714(1-3):148-156, 2013
41. Khalili H, Niakan A, Ghaffarpasand F: Effects of cerebrolysin on functional recovery in patients with severe disability after traumatic brain injury: A historical cohort studh. Clin Neurol Neurosurg 152:34-38, 2017
42. Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vavvaro AR: Pathophysiology and pharmacologic treatment of acute spinal cord injury. Spine J 4(4):451-464, 2004
43. Ladurner G, Kalvack P, Moessler H, Cerebrolysin study group: Neuroprotective treatment with cerebrolysin in patients with acute stroke: A randomised controlled trial. J Neural Transm (Vienna) 112(3):415-428, 2005
44. Lavicky J, Raskova H: Different stressors and blood lipid peroxidation. Arzneimittelforschung 41(8):793-796, 1991
45. Loane DJ, Faden AI: Neuroprotection for traumatic brain injury: Translational challenges and emerging therapeutic strategies. Trends Pharmacol Sci 31(12):596-604, 2010
46. Marsh HN, Palfrey HC: Neurotrophin-3 and brain-derived neurotrophic factor activate multiple signal transduction events but are not survival factors for hippocampal pyramidal neurons. J Neurochem 67(3):952-963, 1996

47. Masliah E, Diez-Tejedor E: The pharmacology of neurtophic treatment with cerebrolysin: Brain protection and repair to counteract pathologies of acute and chronic neurological disorders. Drugs Today (Barc) 48 Suppl A:3-24, 2012
48. Menon PK, Muresanu DF, Sharma A, Mössler H, Sharma HS: Cerebrolysin, a mixture of neurotrophic factors induces marked neuroprotection in spinal cord injury following intoxication of engineered nanoparticles from metals. CNS Neurol Disord Drug Targets 11(1):40-49, 2012
49. Morales-Medina JC, Griffiths NH, Flores G, Mastranzo VM, Iannitti T: Cerebrolysin reduces mechanical allodynia in a rodent model of peripheral inflammation. Neurosci Lett 642:27-30, 2017
50. Muresanu DF, Sharma A, Patnaik R, Menon PK, Mössler H, Sharma HS: Exacerbation of blood-brain barrier breakdown, edema formation, nitric oxide synthase upregulation and brain pathology after heat stroke in diabetic and hypertensive rats. Potential neuroprotection with cerebrolysin treatment. Int Rev Neurobiol 146:83-102, 2019
51. Park YK, Yi HJ, Choi KS, Lee YJ, Kim DW, Kwon SM: Cerebrolysin for the treatment of aneurysmal subarachnoid hemorrhage in adults: A retrospective chart review. Adv Ther 35(12):2224-2235, 2018
52. Patockova J, Krsiak M, Tumova PME: Cerebrolysin inhibits lipid peroxidation induced by insulin hypoclycemia in the brain and heart of mice. Physiol Res 52(4):455-460, 2003
53. Prajda N, Weber G: Malignant transformation-linked imbalance: Decreased Xanthine oxidase activity in hepatomas. FEBS Lett 59(2):245-249, 1975
54. Ren JM, Sietsma D, Qiu S, Moessler H, Finklestein SP: Cerebrolysin enhances functional recovery following focal cerebral infarction in rats. Restor Neurol Neurosci 25(1):25-31, 2007
55. Saeed SA, Shad KF, Saleem T, Javed F, Khan MU: Some new prospects in the understanding of the molecular basis of the patgohenesis of stroke. Exp Brain Res 182(1):1-10, 2007
56. Safi HJ, Hess KR, Randel M, Iliopoulos DC, Baldwin JC, Mootha RK, Shenaq SS, Sheinbaum R, Greene T: Cerebrospinal fluid drainage and distal aortic perfusion: Reducing neurologic complications in repair of thoracoabdominal aortic aneurysm types I and II. J Vasc Surg 23(2):223-228; discussion 229, 1996
57. Sakurai M, Nagata T, Ave K, Horinouchi T, Itoyama Y, Tabayashi K: Survival and death-promoting events after transient spinal cod ischemia in rabbits: Induction of Akt and caspase-3 in motor neurons. J Thorac Cardiovasc Surg 125(2):370-377, 2003
58. Sanli AM, Serbes G, Sargon MF, Calişkan M, Kilinç K, Bulut H, Sekerci Z: Methothrexate attenuates early neutrophil infiltration and the associated lipid peroxidation in the injured spinal cord but does not induce neurotoxicity in the uninjured spinal cord in rats. Acta Neurochir (Wien) 154(6):1045-1054, 2012
59. Schmidley JW: Free radicals in central nervous system ischemia. Stroke 21(7):1086-1090, 1990
60. Schwab ME, Bartholdi D: Degeneration and regeneration of axons in the lesioned spinal cord. Physiol Rev 76(2):319-370, 1996
61. Sharma HS: A select combination of neurotrophins enhances neuroprotection and functional recovery following spinal cord injury. Ann N Y Acad Sci 1122:95-111, 2007
62. Sharma HS: Neurotrophic factors in combination: A possible new therapeutic strategy to influence pathophysiology of spinal cord injury and repair mechanisms. Curr Pharm Des 13(18):1841-1874, 2007
63. Sharma HS: New perpectives for the treatment options in spinal cord injury. Expert Opin Pharmacother 9(16):2773-800, 2008
64. Sharma HS: Selected combination of neurotrophins potentiate neuroprotection and functional recovery following spinal cord injury in the rat. Acta Neurochir Suppl 106:295-300, 2010
65. Sharma HS, Johanson CE: Intracerebroventricularly administered neurotrophins attenuate blood cerebrospinal fluid barrier breakdown and brain pathology following whole-body hyperthermia: An experimental study in the rat using biochemical and morphological approaches. Ann N Y Acad Sci 1122:112-129, 2007
66. Sharma HS, Lundstedt T, Flardh M, Skottner A, Wiklund L: Neuroprotective effects of melanocortins in CNS injury. Curr Pharm Des 13(19):1929-1941, 2007
67. Sharma HS, Meinzingen SZ, Sharma A, Johanson CE: Cerebrolysin attenuates blood-brain barrier and brain pathology following whole body hyperthermia in the rat. Acta Neurochir Suppl 106:321-325, 2010
68. Sherif RN: Effect of cerebrolysin on the cerebellum of diabetic rats: An immunohistochemical study. Tissue Cell 49(6):726-733, 2017
69. Svensson LG, Crawford ES, Hess KR, Coselli JS, Safi HJ: Experience with 1509 patients undergoing thoracoabdominal aortic operations. J Vasc Surg 17(2):357-368; discussion 368-370, 1993
70. Taoka Y, Okajima K, Uchiba M, Murakami K, Kushimoto S, Johno M, Naruo M, Okabe H, Takatsuki K: Role of neurtophils in spinal cord injury in the rat. Neuroscience 79(4):1177-1182, 1997.
71. Tokumine J, Kakinohana O, Cizkova D, Smith DW, Marsala M: Changes in spinal GDNF, BDNF, and NT-3 expression after transient spinal cord ischemia in the rat. J Neurosci Res 74(4):552-561, 2003
72. Tolkovsky A: Neutrophic factors in action-new dogs and new tricks. Trends Neurosci 20(1):1-3, 1997
73. Umehara S, Goyagi T, Nishikawa T, Tobe Y, Masaki Y: Esmolol and landiolol, selsctive beta-1 adrenoreceptor antagonists, provide neuroprotection against spinal cord ischemia and reperfusion in rats. Anesth Analg 110(4):1133-1137, 2010
74. Ustun ME, Duman A, Ogun CO, Vatansev H, Ak A: Effects of nimodipine and magnesium sulfate on endogenous antioxidant levels in brain tissur after experimental head trauma. J Neurosurg Anesthesiol 13(3):227-232, 2001
75. Winterbourn CC, Carr AC: Myeloperoxidase-dependent loss of malondialdehyde: A limitation for detecting neurtophil-mediated lipid peroxidation. Arch Biochem Biophys 302(2):461-467, 1993
76. Wong GKC, Zhu XL, Poon WS: Beneficial effect of cerebrolysin on moderate an severe head injury patients: Result of a cohort study. Acta Neurochir Suppl 95:59-60, 2005

77. Xing S, Zhang J, Dang C, Liu G, Zhang Y, Li J, Fan Y, Pei Z, Zeng J: Cerebrolysin reduces amyloid-β deposits, apoptosis and autophagy in the thalamus and improves functional recovery after cortical infarction. J Neurol Sci 337(1-2):104-111, 2014

78. Yamauchi T, Sawa Y, Sakurai M, Hiroshi T, Matsumiya G, Abe K, Matsuda H: ONO-5046 attenuation of delayed motor neuron death and effect on the induction of brain-derived neurotrophic factor, phosphorylated extracellular signal-regulated kinase, and caspase3 after spinal cord ischemia in rabbits. J Thorac Cardiovasc Surg 131(3):644-650, 2006

79. Yilmaz ER, Kertmen H, Dolgun H, Gurer B, Sanli AM, Kanat MA, Arikok AT, Bahsi SY, Erguder BI, Sekerci Z: Effects of darbepoetin-α in spinal cord ischemia-reperfusion injury in the rabbit. Acta Neurochir (Wien) 154(6):1037-143, 2012

80. Yu Q, Huang J, Hu J, Zhu H: Advance in spinal cord ischemia reperfusion injury: Blood-spinal cord barrier and remote ischemic preconditioning. Life Sci 154:34-38, 2016

81. Zhang C, Chopp M, Cui Y, Wang L, Zhang R, Zhang L, Lu M, Szalad A, Doppler E, Hitzl M, Zhang ZG: Cerebrolysin enhances neurogenesis in the ischemic brain and improves functional outcome after stroke. J Neurosci Res 88(15):3275-3281, 2010

82. Zhang D, Dong Y, Chen J, Wang J, Hou L: Efficacy and safety of cerebrolysin for acute ischemic stroke: A meta-analysis of randomize controlled trials. Biomed Res Int 2017:4191670, 2017

83. Zhang L, Chopp M, Wang C, Zhang Y, Lu M, Zhang T, Zhang ZG: Prospective, double blinded, comparative assessment of the pharmacological activity of Cerebrolysin and distinct peptide preparations for the treatment of embolic stroke. J Neurol Sci 398:22-26, 2019

84. Zhang Y, Chopp M, Zhang ZG, Zhang Y, Zhang L, Lu M, Zhang T, Winter S, Doppler E, Brandstäetter H, Mahmood A, Xiong Y: Cerebrolysin reduces astrogliosis and axonal injury and enhances neurogenesis in rats after closed head injury. Neurorehabil Neural Repair 33(1):15-26, 2019

85. Zhu P, Li J, Fujino M, Zhuang J, Li XK: Development and treatments of inflammatory cells and cytokines in spinal cord ischemia-reperfusion injury. Mediators Inflamm 2013:701970, 2013