Neuroprotective Effects of Niacin on Ischemia/Reperfusion Injury of the Rabbit Spinal Cord

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■ OBJECTIVE: **Previous studies have shown niacin has neuroprotective effects on the central nervous system. However, its specific effect on spinal cord ischemia/reperfusion injury has not yet been explored. This study aims to evaluate whether niacin can contribute neuroprotective effects on spinal cord ischemia/reperfusion injury.**

■ METHODS: **Rabbits were randomized into 4 groups of 8 animals: group I (control), group II (ischemia), group III (30 mg/kg methylprednisolone, intraperitoneal), and group IV (500 mg/kg niacin, intraperitoneal). The rabbits in group IV were premedicated with niacin for 7 days prior to inducing ischemia/reperfusion injury. The control group was subjected only to a laparotomy, while the remaining groups underwent spinal cord ischemia through a 20-minute occlusion of the aorta caudal to the left renal artery. Following the procedure, levels of catalase, malondialdehyde, xanthine oxidase, myeloperoxidase, and caspase-3 were analyzed. Ultrastructural, histopathological, and neurological evaluations were also performed.**

■ RESULTS: **Spinal cord ischemia/reperfusion injury resulted in increased levels of xanthine oxidase, malondialdehyde, myeloperoxidase, and caspase-3, with a concomitant decrease in catalase levels. Treatment with methylprednisolone and niacin led to decreased levels of xanthine oxidase, malondialdehyde, myeloperoxidase, and caspase-3 and an increase in catalase. Both methylprednisolone and niacin treatments demonstrated improvements in histopathological, ultrastructural, and neurological assessments.**

■ CONCLUSIONS: **Our findings suggest that niacin has antiapoptotic, anti-inflammatory, antioxidant, and neuroprotective effects at least equal to methylprednisolone in ischemia/reperfusion injury of the spinal cord. This study is the first to report the neuroprotective impact of niacin on spinal cord ischemia/reperfusion injury. Further research is warranted to elucidate the role of niacin in this context.**

INTRODUCTION

Spinal cord ischemia/reperfusion (I/R) injury is a devastating and unpredictable complication that can occur following thoracoabdominal surgery, potentially leading to paraplegia.[1] Neuronal injury is a consequence of primary injury or secondary injury. The primary injury, which is irreversible, occurs at the time of ischemia. Upon reperfusion, secondary injury mechanisms are activated, including apoptosis,

Key words
- Ischemia/reperfusion
- Methylprednisolone
- Neuroprotection
- Niacin
- Spinal cord

Abbreviations and Acronyms
CAT: Catalase
ELISA: Enzyme-linked immunosorbent assay
FOR: Free oxygen radical
H_2O_2: Hydrogen peroxide
I/R: Ischemia/reperfusion
MCA: Middle cerebral artery
MDA: Malondialdehyde
MP: Methylprednisolone
MPO: Myeloperoxidase
nM: Nanomoles
TBA: Thiobarbituric acid
TEM: Transmission electron microscope
XO: Xanthine oxidase

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inflammation, the formation of free oxygen radicals (FORs), and lipid peroxidation.[2-7]

Niacin, a water-soluble vitamin essential for the metabolism of carbohydrates, proteins, and fats, also serves as a building block for nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate. These components are crucial for glycolysis, fatty acid biosynthesis, citrate conversion, and respiratory chain enzyme activity.[8] Niacin has varied effects on body tissues, including the central nervous system. It has been demonstrated that niacin has beneficial effects in the treatments of dyslipidemia, hypercholesterolemia, type 2 diabetes mellitus, obesity, atherosclerosis, hyperalgesia, lung and kidney damage, neurodegenerative disorders (e.g., Alzheimer disease, Parkinson disease, Huntington disease), and psychological disorders (e.g., depression, anxiety, schizophrenia, bipolar disorder, psychological distress).[9-18] Niacin has neuroprotective effects on the brain in I/R injury. Chen et al. showed that niacin had neuroprotective effects in cerebral ischemia. In cerebral ischemia caused by middle cerebral artery (MCA) occlusion, niacin provides neovascularization by increasing vascular endothelial growth factor, endothelial nitric oxide synthase, and angiopoietin-1 levels, thus reducing neurological losses.[19] A study of the use of niacin in cerebral ischemia resulting from MCA occlusion showed that niacin increases local cerebral blood flow, triggers arteriogenesis, and increases vascular smooth muscle cell proliferation.[20] Niacin also has neuroprotective effects on traumatic brain injury. Previous studies have demonstrated that administering niacin treatment following traumatic brain injury improves behavioral performance and reduces lesion sizes.[21,22]

The neuroprotective effects of substances such as testosterone, vitamin D, ganoderma lucid polysaccharides, dexpanthenol, darbopoetin, and mesna on spinal cord I/R injury have been previously studied.[2-7] However, no previous study has examined the neuroprotective effects of niacin after spinal cord I/R injury. This study aims to evaluate whether niacin has neuroprotective activity on the spinal cord in I/R injury in rabbits. Methylprednisolone (MP) is widely used in spinal cord I/R injuries. For this reason, we compared the effects of niacin with MP.[23,24]

MATERIALS AND METHODS

Experimental Groups

Animal care and all experiments were conducted in adherence to the European Communities Council Directive of September 22, 2010, 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 Saki Yenilli Experimental Animal Production and Practice Laboratory Local Ethics Committee (26.06.2020–03/13). Thirty-two male adult New Zealand white rabbits, weighing 2800–3750 g, were randomly separated into the following 4 groups (8 rabbits in each group):

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

Group 2: Ischemia group (n = 8); exposure to transient global spinal cord ischemia. The occlusion clamp was released, and the same volume of saline (2 cc 0.9% NaCl) was infused intraperitoneally. Rabbits underwent laminectomy, and spinal cord samples were taken 24 hours after ischemia.

Group 3: MP group (n = 8); treatment similar to group 2, but a single dose of 30 mg/kg MP (Prednol, Mustafa Nevzat, Turkey) was administered intravenously to these rabbits immediately after occlusion clamp removal. The MP dose was determined according to previous studies.[2-7,25]

Group 4: Niacin group (n = 8); treatment with 500 mg/kg niacin intraperitoneally for 7 days preoperatively. One hour after the niacin injection on the seventh day, the same surgical procedure was performed to the rabbits in group 2. The niacin dose was determined according to previous studies.[26-29]

Anesthesia and Surgical Procedures

Rabbits were fed standard chow and water *ad libitum* at ideal room temperature (18°C–21°C) and maintained under a 12-h light/12-h dark cycle. They were anesthetized intramuscularly with 5 mg/kg xylazine (Rompun, Bayer, Turkey) and 70 mg/kg ketamine (Ketalar, Parke Davis Eczacıbası, Turkey) and were allowed to respire spontaneously. An anal thermometer was used to measure body temperature (Digital Fever thermometer, Becton Dickinson, NJ, USA), maintained at 37°C with a warming cushion. Surgery was performed with animals in the supine position. After sterilization and a 10-cm midline incision, the abdominal aorta was approached transperitoneally. For anticoagulation, 150 U/kg heparin (Nevparin, Mustafa Nevzat, Turkey) was administered intravenously 5 minutes before clamping. With the aim of surgical microscope, an aneurysm clip with a closing force of 70 g was applied to the aorta approximately 1 cm under the renal artery (Yasargil, FE721, Aesculap, Germany). Twenty minutes of cross-clamping was applied. After the occlusion period was completed, the clamps were removed, and blood flow restoration was confirmed visually.

The rabbit aortic cross-clamping technique used in this study is an established and valuable system with the 20-minutes ischemia period providing satisfactory damage.[2-7] Rabbits were allowed free access to food and water 2 hours after surgery. Crede maneuver was administered at least twice a day to rabbits who developed neurogenic bladder. All rabbits were sacrificed by the infusion of high-dose pentobarbital (200 mg/kg) (Nembutal, Oak Pharmaceuticals, Lake Forest, IL, USA) 24 hours after surgery. The spinal cord segment between L2 and L5 levels was removed by total laminectomy for biochemical, histopathological, and ultrastructural examination.

For the biochemical examination, blood (10 cm^3) was taken from the left ventricle and was centrifuged at 1000 × g for 5 minutes, and the clear supernatant was analyzed. All serum and tissue test samples were kept at −80°C for further analysis; at that point, the tissue was homogenized (B. Braun Melsungen AG 853202, Melsungen, Germany) in physiologic saline (1/5w/v) and centrifuged at 1780 × g for 20 minutes. The protein level of

the clear supernatant then was studied by Lowry method, and the concentrations equalized before analysis. Serum samples from the upper clear supernatant of centrifuged blood were used for the biochemical examination. The study design is summarized in Figure 1.

Biochemical Procedures

Serum and Tissue Caspase-3 Activity. Caspase-3 concentration was evaluated by enzyme-linked immunosorbent assay (ELISA) (ELISA kit; Cusabio, Hubei, China), a technique that uses the quantitative sandwich protein immunoassay. ELISA was performed according

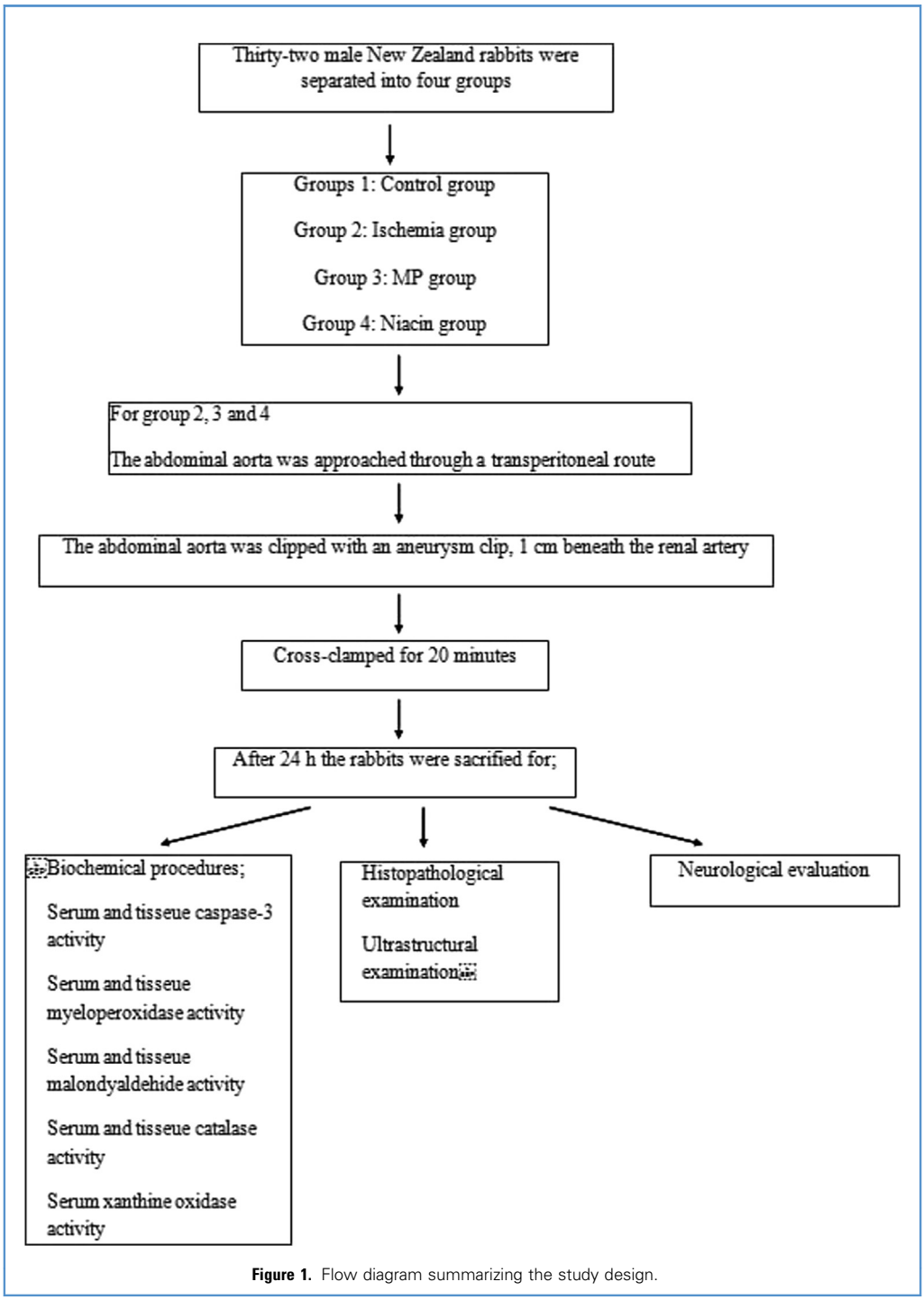

Figure 1. Flow diagram summarizing the study design.

to manufacturer's guidelines. A microplate was precoated with antibodies specific for caspase-3. Standards and samples were pipetted into wells allowing precoated antibodies to bind caspase-3 present in samples and standards, if any. After removing unbound substances, a biotin-conjugated antibody specific for caspase-3 was added to the wells. Avidin-conjugated horseradish peroxidase was added to the wells after washing. Unbound substances were removed via 3 washes with a washing buffer before the avidin protein reagent was added to the wells. The intensity of the color that developed was commensurate to the concentration of caspase-3 bound in the initial step. The intensity of the color was measured at 450 nm when color development stopped. Caspase-3 concentrations were determined by comparing the absorbance values of the samples with those of standard caspase-3 solutions. The results are presented in nanograms per milliliter.

Serum and Tissue Myeloperoxidase Activity. Myeloperoxidase (MPO) activity was determined by competitive inhibition ELISA (Cusabio, Hubei, China) in accordance with manufacturer's instructions. The provided microtiter plate was precovered with an MPO-specific antibody. Standards or samples were added to appropriate microtiter plate wells with biotin-conjugated MPO, and a competitive inhibition reaction was started between MPO (from standards or samples) and biotin-conjugated MPO with the precoated MPO-specific antibody. With greater amounts of MPO, lower amounts of antibodies are bound by biotin-conjugated MPO. After washing, avidin-conjugated horseradish peroxidase was added to the wells. The substrate solution was then added, and the color was allowed to develop to show the amount of MPO in the sample. The intensity of the color was measured at 450 nm at the point that color development stopped. MPO activities were computed by comparing the absorbance estimates of the samples to those of the standard MPO solutions. Results are expressed in nanograms per milliliter.

Serum and Tissue Malondialdehyde Activity. Serum malondialdehyde (MDA) levels were measured using thiobarbituric acid (TBA). Specimens were mixed with 2 volumes of cold saline liquid containing 0.07% sodium dodecyl sulfate and 0.001% butylated hydroxytoluene. At that point, 1 mL of the samples was added to 500 μL of TBA with 0.01 μL of NH_2SO_4 (0.67% TBA in half-acidic corrosive) to precipitate proteins.

The specimens were heated in boiling water for 1 hour, cooled, and blended with an equivalent volume (2 mL) of n-butanol. The mixture was centrifuged at 1780 × g at room temperature for 10 minutes. The absorbance of the organic layer was determined at 535 nm in a 1 mL cell (Molecular Devices Corporation, Sunnyvale, CA, USA). The MDA levels were calculated by comparing the absorbance values of the samples with those of the standard MDA solutions and are presented in nanomoles.

Serum and Tissue Catalase Activity. Catalase (CAT) levels were evaluated by measuring the rate of absorbance decrease of hydrogen peroxide (H_2O_2) at 240 nm.[30] H_2O_2 extinction coefficients were used to calculate CAT levels. Results are presented in International Units per milliliter.

Serum Xanthine Oxidase Activity. Serum xanthine oxidase (XO) activity was determined with the Prajda/Weber technique, where activity is measured by the determination of the amount of uric acid formed from xanthine.[25] Serum samples (100 μL) were incubated at 37°C in 3 mL of phosphate buffer (pH 7.5, 50 nM) containing xanthine (4 mM) for 30 minutes. The reaction was halted by adding 0.1 mL of 100% (w/v) trichloroacetic acid, and the mixture was then centrifuged at 1780 × g for 20 minutes. Uric acid levels were measured in the supernatant by measuring absorbance at 292 nm against a blank and are presented in milli-international units per milliliter. A calibration curve was constructed using 10–50 mU/mL concentrations of standard XO solutions (Sigma X-1875, Sigma-Aldrich, St. Louis, MO). One unit of activity was defined as 1 μmol of uric acid formed per minute at 37°C and pH 7.5.

Histopathological Procedures

Spinal cord samples were taken 24 hours after injury for histological evaluation. Each cord section was immersed in 4% paraformaldehyde with 0.1 mol/l phosphate buffer and maintained at 4°C. The samples were embedded in paraffin, cut into 5-μm thick sections, and stained with hematoxylin and eosin. The samples were examined under a light microscope by a neuropathologist who was blinded to the study plan. Five distinct areas of spinal cord gray matter were evaluated using a 40× objective. A semi-quantitative scoring system ranging from 0 to 3 was used to appraise histopathological changes in the spinal cord tissue of all samples. Six different parameters (hemorrhage, congestion, necrosis, edema, neuronal loss, and inflammation) were histopathologically appraised and scored as follows: 0 = negligible, 1 = mild, 2 = moderate, and 3 = common. The histopathology score for every spinal cord sample was determined by averaging the scores of these 6 parameters.[2-7] Also, a point-by-point appraisal of the level of ischemic neuronal injury was similarly performed. The quantity of typical motor neurons in the anterior horn of the spinal cord (anterior to a line drawn through the central canal perpendicular to the vertebral axis) was determined, and for every rabbit, 3 fields were estimated using a 40× objective. A mean normal motor neuron count for the fields from every rabbit was determined. Neurons containing Nissl substance, loose chromatin, and prominent nucleoli were determined normal and viable.[31]

Ultrastructural Analysis

Tissue samples were cleared of blood with a scalpel, and the meninges were cautiously removed. The tissue samples were fixed in 2.5% glutaraldehyde for 24 hours, then washed in phosphate buffer (pH: 7.4). They were postfixed for 2 hours in 1% osmium tetroxide in phosphate buffer (pH: 7.4) and dehydrated with increasing concentrations of alcohol, then washed with propylene oxide and embedded in epoxy resin media. Semithin sections, almost 2-mm thick, and ultrathin sections, almost 60-nm thick, were cut with a LKB-Nova ultramicrotome (LKB- Produkter AB, Bromma, Sweden) glass knife. The semithin sections were stained with methylene blue and evaluated with a Nikon Optiphot (Nikon Corporation, Tokyo, Japan) light microscope. Then the tissue blocks were trimmed, and the ultrathin sections were prepared using the same ultramicrotome. These sections were stained with lead citrate and uranyl acetate. Following staining, all the ultrathin sections were examined using a Jeol JEM 1200 EX (Jeol Ltd.,

Tokyo, Japan) transmission electron microscope (TEM), and electron micrographs were taken by the same TEM at 5000x magnification. A total of 100 large-sized myelinated axons, 100 medium-sized myelinated axons, and 100 small-sized myelinated axons were evaluated for every sample. They were scored from 0 to 3 and counted. The data are presented in terms of mean values, as described by Kaptanoglu et al.[32]

The scoring system was as follows:

0: Ultrastructurally normal myelinated axon.

1: Separation in myelin configuration.

2: Interruption in myelin configuration.

3: Honeycomb appearance in myelin configuration.

Neurologic Evaluations

The neurological function of the hindlimbs 24 hours after the procedure was evaluated to score the neurological status of the animals; the modified Tarlov scoring system was used.[33] A score from 0 to 5 was assigned to each animal:

0 = No voluntary hindlimb movement.

1 = Perceptible movement of joints.

2 = Active movement but unable to sit without assistance.

3 = Able to sit but unable to hop.

4 = Weak hop.

5 = complete recovery of hindlimb function.

A medical doctor blinded to the experimental groups performed the neurologic evaluations.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 22 (IBM SPSS, Türkiye). The Kolmogorov-Smirnov and Shapiro-Wilk tests assessed the normality of the distribution of continuous variables. Medians were compared with Kruskal-Wallis test. If the P value obtained from the Kruskal-Wallis test was statistically significant, the differences between the groups were evaluated with Dunn significant difference test and Kruskal-Wallis nonparametric comparison tests. P values less than 0.05 were determined statistically significant.

RESULTS

Serum and Tissue Caspase-3 Analysis

When the serum and tissue caspase-3 activities of the control and ischemia groups were compared statistically, a significant increase was detected in serum and tissue caspase-3 activities in the ischemia group ($P = 0.011$ and $P = 0.006$). When the MP group was compared with the ischemia group, a statistically significant decrease was detected in serum and tissue caspase-3 activities in the MP group ($P = 0.002$ and $P = 0.006$). When the niacin group was compared with the ischemia group, as in the MP group, a statistically significant decrease was found in serum and tissue caspase-3 activities ($P = 0.046$ and $P = 0.001$). No significant difference was found between the niacin and MP groups ($P = 0.63$ and $P = 0.871$) (Figure 2A and B).

Serum and Tissue MPO Analysis

There were significant differences between the control and ischemia groups in their mean serum and tissue MPO activities ($P < 0.001$ and $P = 0.031$). When the MP group was compared with the ischemia group, the serum and tissue MPO activities of the MP group were found to have statistically significantly decreased ($P = 0.038$ and $P = 0.001$). When the niacin group was compared with the ischemia group, the serum and tissue MPO activities of the niacin group were found to be statistically significantly lower than those of the ischemia group ($P = 0.014$ and $P < 0.001$). There was no statistically significant difference between the niacin and MP groups in terms of serum and tissue MPO activities ($P = 0.682$ and $P = 0.72$) (Figure 2C and D). In the ischemia group, serum and tissue MPO activities seem to increase as an indicator of increased inflammatory response. Serum and tissue MPO activities decreased after niacin and MP treatments.

Serum and Tissue MDA Analysis

Significant differences were seen between the control and ischemia groups in mean serum and tissue MDA activities ($P = 0.003$ and $P < 0.001$). Serum and tissue MDA activities of the ischemia group were higher than those of the control group. When compared with the ischemia group, a statistically significant decrease was seen in the niacin group, as in the MP group, in serum and tissue MDA activities ($P < 0.001$, for both). There was no statistically significant difference between the niacin and MP groups in serum and tissue MDA activities ($P = 0.079$ and $P = 0.611$) (Figure 2E and F).

Serum and Tissue CAT Analysis

When the serum and tissue CAT activities of the control and ischemia groups were compared, a statistically significant decrease was seen in serum and tissue CAT activities in the ischemia group ($P = 0.028$ and $P = 0.002$). When the MP group was compared with the ischemia group, a statistically significant increase was seen in serum and tissue CAT activities in the MP group ($P = 0.002$ and $P < 0.001$). A statistically significant increase was seen in serum and tissue CAT activities in the niacin group, as detected in the MP group ($P < 0.001$, for both). There was no statistically significant difference between the serum and tissue CAT activities of the niacin and MP groups ($P = 0.84$ and $P = 0.623$) (Figure 3A and B).

Serum XO Analysis

When serum XO activity of the control and ischemia groups was compared, a statistically significant increase in serum XO activity was seen in the ischemia group ($P = 0.02$). When the serum XO activities of the MP and niacin groups separately were compared with the ischemia group, a statistically significant decrease was seen for both ($P = 0.001$ and $P < 0.001$). When the niacin group and the MP group were compared, no statistically significant difference was seen for serum XO activity ($P = 0.578$) (Figure 3C).

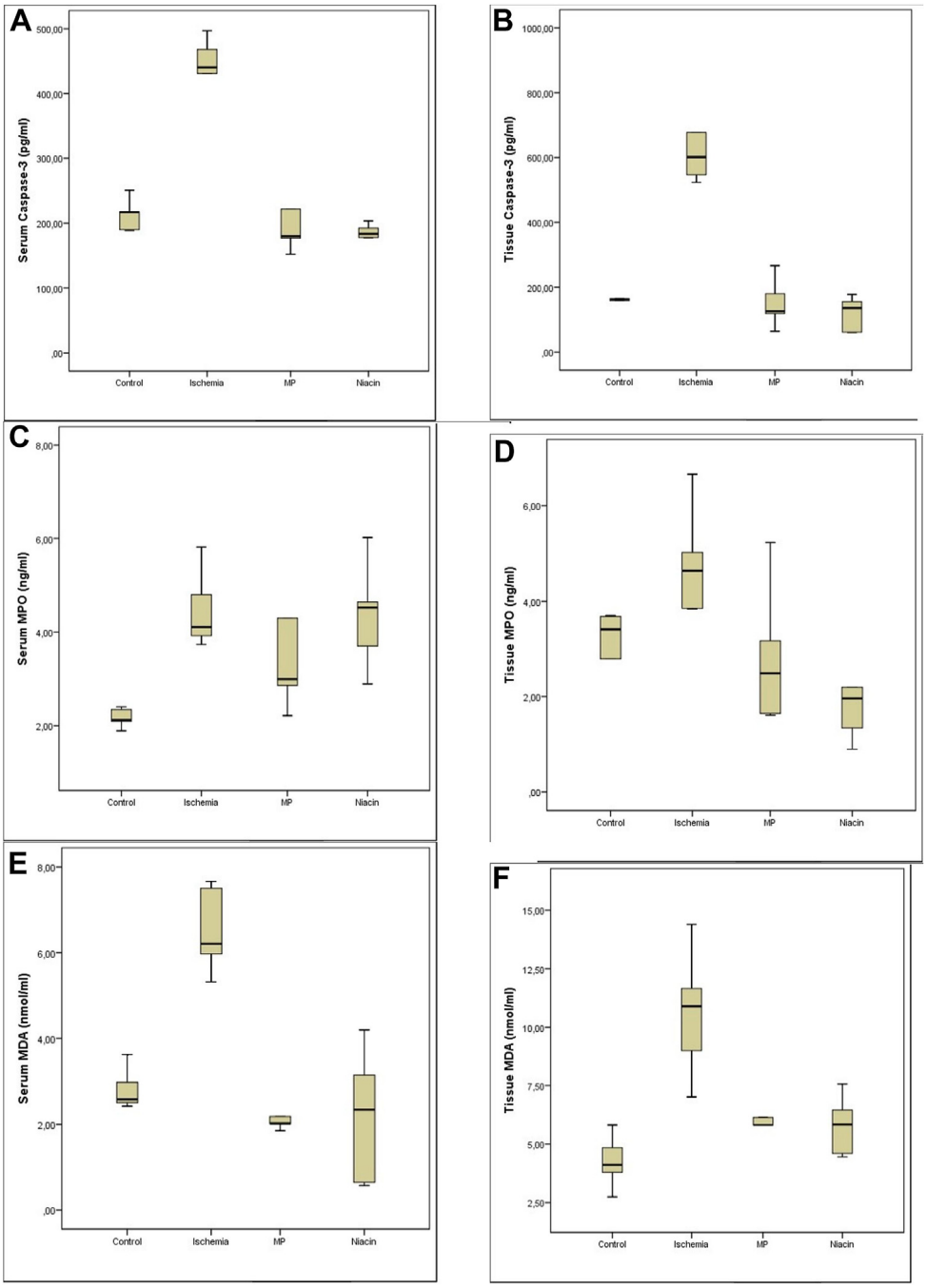

Figure 2. Box and whisker plots of levels of key markers of ischemia/reperfusion injury. (**A**) Serum caspase-3 activities*, (**B**) Tissue caspase-3 activities*, (**C**) Serum MPO activities*, (**D**) Tissue MPO activities*, (**E**) Serum MDA activities*, and (**F**) Tissue MDA activities*. *: The arithmetic mean is indicated by the box in the middle of each whisker; the whiskers at the top and bottom of the box show +1 SD and −1 SD, respectively. **: The median is indicated by the horizontal lines in the middle of each box; the upper and lower bounds of the box represent the 25th and 75th percentiles, respectively. The highest and lowest levels observed are indicated by whiskers at the top and bottom of the box. MDA, malondialdehyde; MP, methylprednisolone; MPO, myeloperoxidase; SD, standard deviation.

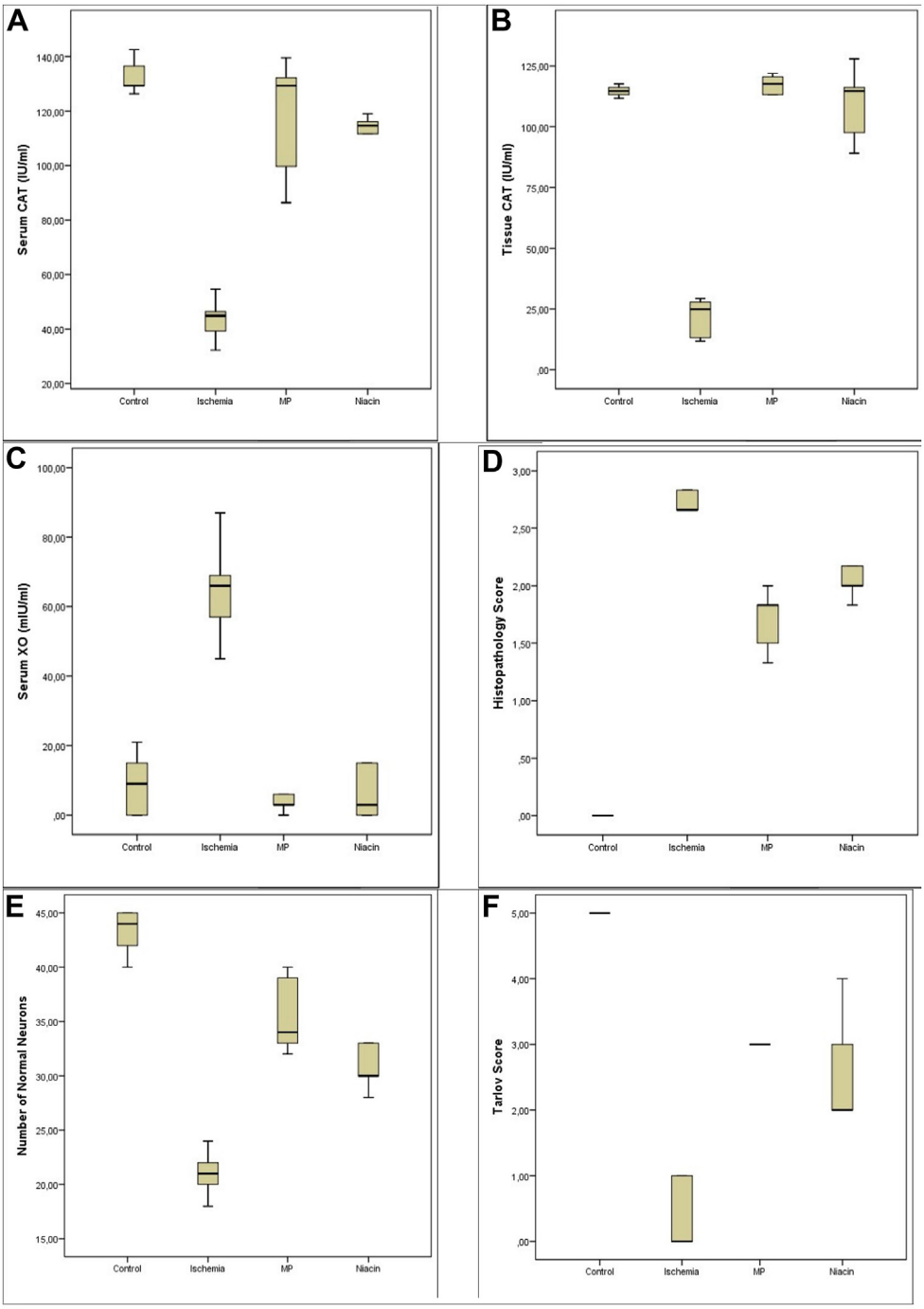

Figure 3. Box and whisker plots of tissue levels of key markers of ischemia/reperfusion injury. (**A**) Serum CAT activities*, (**B**) Tissue CAT activities*, (**C**) Serum XO activities*, (**D**) Histopathology score graphic**, (**E**) Normal neuron number graphic*, and (**F**) Tarlov score graphic**. *: The arithmetic mean is indicated by the box in the middle of each whisker; the whiskers at the top and bottom of the box show +1 SD and −1 SD, respectively. **: The median is indicated by the horizontal lines in the middle of each box; the upper and lower bounds of the box represent the 25th and 75th percentiles, respectively. The highest and lowest levels observed are indicated by whiskers at the top and bottom of the box. CAT, catalase; XO,xanthine oxidase; SD, standard deviation.

Histopathological Procedures

Light microscopy examinations found spinal cord samples from the control group to be normal (Figure 4A). Samples taken from the ischemia group 24 hours after I/R injury showed diffuse hemorrhage and gray matter congestion, with marked necrosis and diffuse edema in white and gray matter. Lymphocytes, plasma cells, and infiltrated polymorphonuclear leukocytes were observed in the damaged areas, and cytoplasmic eosinophilia, neuronal pyknosis, and loss of cytoplasmic features were also seen in the ischemia group (Figure 4B). Cord tissues were seen to be well protected from I/R injury in the niacin group and the MP group (Figure 4C and D).

When the pathological scores of the control and ischemia groups were compared, the ischemia group score was found to be statistically significantly higher ($P = 0.006$). The pathological score of the MP group was statistically significantly lower than that of the ischemia group ($P = 0.023$). When the niacin group was compared with the ischemia group, a significantly lower pathological score was observed ($P = 0.002$). When the histopathological scores of the niacin and MP groups were compared, no statistically significant difference was seen ($P = 0.695$) (Figure 3D). These data indicate that histopathologically, both niacin and MP protect the spinal cord from I/R injury.

Compared to the control group, the number of normal motor neurons in the anterior spinal cord of the ischemia group was statistically significantly less ($P = 0.008$). Compared to the ischemia group, the number of normal motor neurons in the anterior spinal cord was found to be statistically significantly higher in the MP group ($P = 0.029$). The niacin group had a statistically significantly higher number of normal motor neurons in the anterior spinal cord, as in the MP group ($P = 0.048$). When the number of normal motor neurons in the anterior spinal cord of the niacin group and the MP group was compared, no statistically significant difference was found ($P = 0.74$) (Figure 3E). It appears that histopathologically, both niacin and MP significantly protect the spinal cord from I/R injury.

Ultrastructural Analysis

Ultrastructural pathologic changes were not observed in TEM examinations of gray and white matter of spinal cord tissue samples belonging to the control group. The ultrastructural appearance of the neurons was normal, and there were no pathological changes in intracellular organelles, membranes, nuclei, and perineuronal tissues. However, slight detachments were observed in a small portion of the myelin sheath in a few of the large myelinated axons. This may be due to delayed fixation of the tissue. The

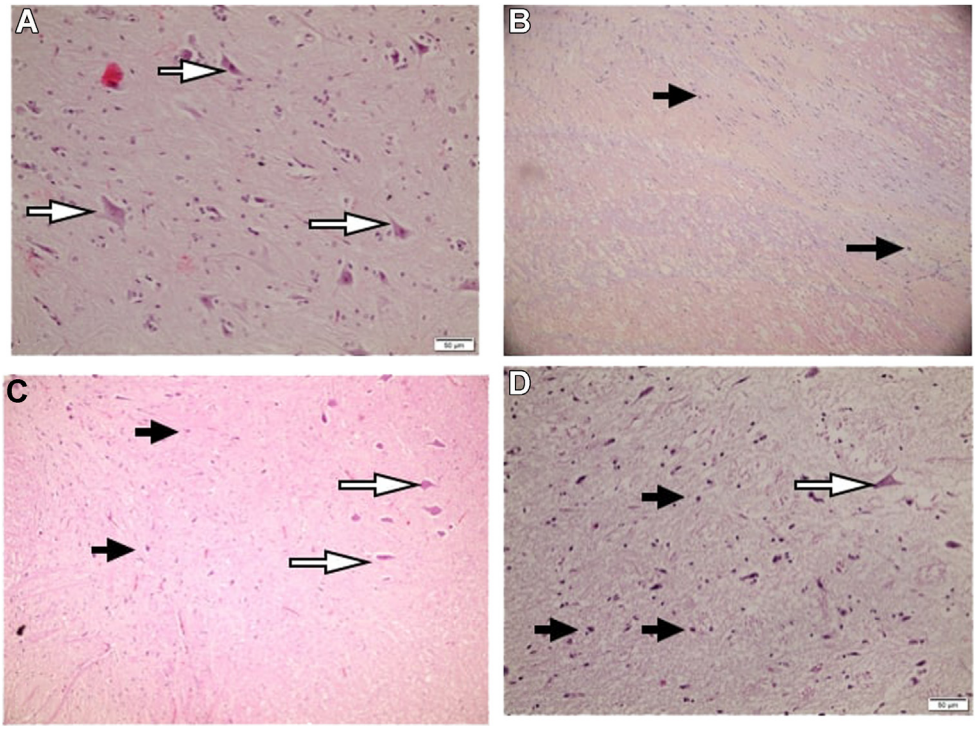

Figure 4. Photomicrographs of 5-μm spinal cord tissue sections obtained from each study group. Images are shown with hematoxylin–eosin staining under a 10× objective. (**A**) Control group has normal spinal cord parenchyma. Normal neurons are shown with arrows. (**B**) Ischemia group, edematous surface has degenerated neurons (*filled arrows*). (**C**) Methylprednisolone group has less degenerated neurons (*filled arrows*) and normal neurons (*hollow arrows*). (**D**) Niacin group has less degenerated neurons (*filled arrows*) and more normal neurons (*hollow arrows*).

remainder of the large myelinated axons and all small and medium myelinated axons were ultrastructurally normal (Figure 5A).

TEM examination revealed vacuoles in the neuron cytoplasm and perineural edema in the ischemia group gray matter. Neuron nuclei and cell membranes appeared normal. White matter TEM examination of spinal cord samples taken from the ischemia group revealed separations in the myelin configuration of small, medium, and large myelinated axons (Figure 5B). Of all the groups, the ischemia group showed the most ultrastructural damage to myelinated axons.

TEM examination of the spinal cord gray matter of the MP group revealed perineural edema and vacuoles in neuronal cytoplasm; the neuronal cell membranes and nuclei appeared normal. TEM examination of the white matter of the MP group revealed myelin configuration differences in small, medium, and large myelinated axons (Figure 5C).

TEM examination of spinal cord samples taken from the niacin group revealed normal neuronal cell membranes, cytoplasm, and nuclei. TEM examination of gray matter revealed widespread perineural edema. TEM examination of the white matter of the niacin group revealed separations in the myelin configuration of small, medium, and large myelinated axons (Figure 5D).

When the TEM examinations of the ischemia and control groups were compared; small, medium, and large-sized myelinated axons were statistically significantly more differentiated in the ischemia group ($P < 0.001$, $P = 0.005$, and $P = 0.006$). When the TEM examinations of the MP and ischemia groups were compared, small myelinated axons were seen to diverge in both groups with no difference in the extent of separation ($P = 0.6$). Medium and large myelinated axons were statistically significantly better preserved in the MP group ($P < 0.001$, for both). When compared with the ischemia group, the niacin group showed no statistical difference in the separation in small myelinated axons ($P = 0.573$), as was true of the MP group; in addition, medium and large myelinated axons were statistically significantly better preserved in the niacin group ($P = 0.002$ and $P = 0.005$). When the MP and the niacin groups were compared, no difference was found in small myelinated axons ($P = 0.81$), but the niacin group's medium and large myelinated axons were statistically significantly better preserved ($P = 0.009$ and $P < 0.001$). The protective effects of niacin and MP treatments on small axons were not detected; we inferred that both treatments, containing more niacin, statistically significantly protected medium and large myelinated axons from I/R injury.

Neurologic Evaluations

The modified Tarlov score of the ischemia group was statistically significantly lower than that of the control group ($P < 0.001$). The mean modified Tarlov scores of both the niacin and MP groups were statistically significantly higher than those of the ischemia group ($P = 0.011$ and $P < 0.001$). There was no statistically significant difference between the mean modified Tarlov scores of the niacin and MP groups ($P = 0.77$) (Figure 3F). Niacin, which has been shown to be neuroprotective biochemically, histopathologically, and ultrastructurally, also contributes to the neurological recovery after I/R injury.

DISCUSSION

Ischemia is a condition in which blood flow to the tissue is insufficient to support cellular functions. Due to their restricted anaerobic metabolism and limited glycogen stores, the central nervous system and spinal cord are among the tissues most susceptible to ischemia. Decreased blood flow and tissue suffusion and the consequent depletion of cellular energy stores initiate a chain of reactions known as the ischemic cascade, inevitably resulting in cell death.[34] Tissue destruction in spinal cord ischemia occurs via primary and secondary injuries. Primary injury occurs at the time of ischemia and is irreversible. With reperfusion, secondary injury occurs through mechanisms that include apoptosis, inflammation, FOR formation, and lipid peroxidation.[2-7]

Paraplegia as a result of spinal cord I/R injury is an unpredictable complication that can occur after descending and thoracoabdominal aorta surgery. Hypoperfusion during aortic cross-clamping causes primary injury to the spinal cord. After the

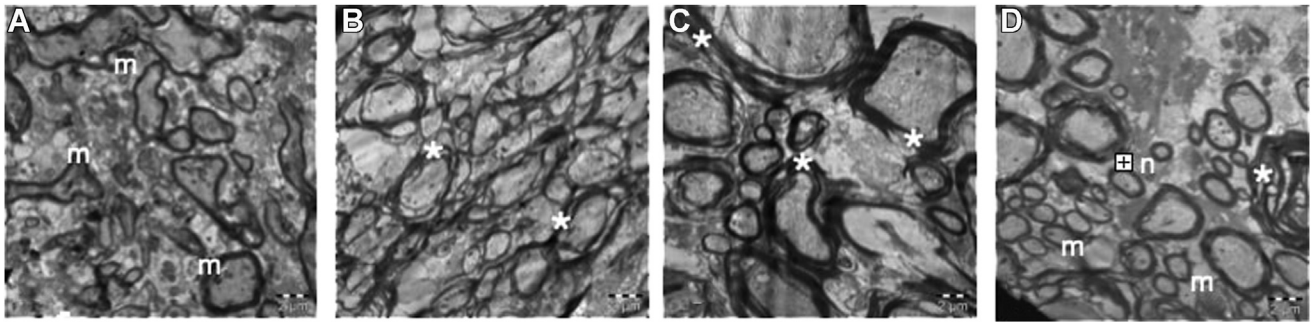

Figure 5. Representative transmission electron micrographs showing each group. (**A**) Control group has ultrastructurally normal myelinated axons (m) (original magnification 5000×, scale bar = 2 μm). (**B**) Ischemia group has small, medium, and large axons with separations in myelin configuration (*) (original magnification = 5000×, scale bar = 2 μm). (**C**) Methylprednisolone group has separations in myelin configurations (*) in medium and large myelinated axons (original magnification = 5000×, scale bar = 2 μm). (**D**) Niacin group has large myelinated axons with separations in myelin configuration (*) and ultrastructurally normal, small myelinated axons (m) and neuronal nuclei (n) (original amplification = 5000×, scale bar = 2 μm).

removal of the clamp, the restoration of blood flow results in secondary injury and ultimately spinal cord dysfunction. Reperfusion injury, which develops with the restoration of blood flow after primary injury, causes the existing damage to increase further.[1]

Niacin is a water-soluble vitamin needed for carbohydrate, protein, and fat metabolism. It is a molecular building block of nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, which are essential for glycolysis, fatty acid biosynthesis, citrate conversion, and respiratory chain enzymatic activity.[8] Niacin produces varied effects on many body tissues, including the central nervous system. It provides benefits for the treatment of dyslipidemia, hypercholesterolemia, type 2 diabetes mellitus, obesity, atherosclerosis, hyperalgesia, lung and kidney damage, neurodegenerative disorders (Alzheimer disease, Parkinson disease, and Huntington disease). and psychological disorders (depression, anxiety, schizophrenia, bipolar disorder, and psychological distress) and is neuroprotective in I/R injury.[9-18] Chen et al. showed that niacin is neuroprotective in the setting of cerebral ischemia. In cerebral ischemia caused by MCA occlusion, niacin promotes neovascularization by increasing vascular endothelial growth factor, endothelial nitric oxide synthase, and angiopoietin-1 levels, reducing neurological losses.[19] A study of the use of niacin in cerebral ischemia resulting from MCA occlusion showed that niacin increases local cerebral blood flow, triggers arteriogenesis, and increases vascular smooth muscle cell proliferation.[20]

The protective efficacy of niacin in I/R injury in different tissues like the brain, lung, kidney, and liver has been shown previously.[26,35,36] However, no previous study has examined the neuroprotective effects of niacin after spinal cord I/R injury. This study evaluated whether niacin is neuroprotective to the spinal cord in I/R injury in rabbits. Because MP is widely used in spinal cord I/R injuries, we used it as a basis for comparison with niacin.[23,24]

Apoptosis is a form of programmed cell death that develops after spinal cord I/R injury and causes neural cell death. Activation of proapoptotic proteins located on the outer surface of mitochondria turn on apoptotic pathways. Apoptotic protease activating factor 1 released by expression of Bax and Bak proteins causes the release of cytochrome c into the cytosol and activates the caspase cascade.[37] Caspases are enzymes from the cysteine protease family that are involved in apoptosis. The main effector of apoptosis is caspase-3, an interleukin-converting enzyme.[38] Previous studies have shown that increased caspase-3 activity is a reliable indicator of apoptosis in spinal cord I/R injury.[2-7] After cerebral I/R injury, c-Jun N-terminal kinases and extracellular signal regulatory kinase 1/2 phosphorylation due to increased apoptosis trigger the apoptotic cascade and increase caspase-3 activity.[39] It has been shown that niacin treatment reduces caspase-3 activity and has antiapoptotic activity.[39,40] This study observed that serum and tissue caspase-3 activities increased in ischemic tissue at the 24th hour following spinal cord I/R injury, indicating increased apoptosis. Both MP and niacin reduce caspase-3 activity in the spinal cord resulting in an antiapoptotic effect and protecting the cord from apoptotic damage.

An important component of secondary injury after spinal cord I/R is the inflammatory response. Neutrophils, macrophages, and monocytes are important mediators of the inflammatory response and are the leading agents of tissue damage after reperfusion. Macrophage and microglia activation, exacerbation of inflammation by causing the release of proinflammatory cytokines and chemokines, accumulation of FOR and arachidonic acid metabolites result in cell damage and cell death.[41] Niacin increases the number of macrophages and increases the inflammatory response.[42]

Concentrated in the azurophilic granules of neutrophils, MPO increases in direct proportion to the activity and number of neutrophils infiltrating the spinal cord and is a reliable indicator of neutrophilic infiltration of injured tissue.[43,44] MPO neutralizes H_2O_2 formed in phagocytic leukocytes resulting from increased FOR formation following ischemia. In previous spinal cord I/R studies, increased serum and tissue MPO activity levels have indicated inflammatory response.[2-7] After I/R injury, MPO activity increases; by reducing MPO activity, niacin exerts anti-inflammatory effects on the brain and other organs.[19,20,26,27,45] Both MP and niacin treatment exert anti-inflammatory effects by reducing MPO activity in tissue and serum.

Ischemia leads to decreased adenosine triphosphate, triggering increased FOR formation. When FOR formation surpasses the antioxidant capacity, it leads to the oxidation of polyunsaturated fatty acids in the cell membrane, initiating lipid peroxidation. Lipid peroxidation is primarily responsible for cell membrane damage that occurs after spinal cord I/R injury. MDA is an end product of lipid peroxidation and is a reliable indicator of peroxidation reactions.[46] Previous lung, kidney, and pancreatic I/R injury studies have shown MDA levels increase, and niacin treatment reduces MDA formation and prevents lipid peroxidation.[26,27,34] This study observed that serum and tissue MDA levels increase as an indicator of increased lipid peroxidation following spinal cord I/R injury, and both MP and niacin treatments reduce MDA levels in the spinal cord. Decreased MDA levels indicate that niacin prevents lipid peroxidation in spinal cord I/R injury.

Oxidative damage is a mechanism causing neuronal loss through excessive FOR production in both ischemia and reperfusion processes. With increased oxidative stress, antioxidant mechanisms in cells are activated.[47] CAT is an antioxidant enzyme that reduces oxidative stress by binding FORs. Where oxidative stress exceeds antioxidant capacity, CAT activity is relatively reduced.[48] In lung and pancreatic I/R injury models, niacin has been shown to have antioxidant activity by reducing oxidative stress.[26,27] This study observed that serum and tissue CAT levels decreased in ischemic spinal cord tissue after I/R injury. After MP and niacin treatments, serum and tissue CAT activities increased, indicating that both drugs had antioxidant activity.

Increased intracellular Ca^{+2} resulting from ischemia damages the cell membrane and activates XO. XO catalyzes the production of superoxide anion. An elevated XO level indicates oxidative stress after spinal cord I/R injury.[46] This study observed that serum XO activity increased, an indication of oxidative stress in ischemic spinal cord tissue after I/R injury. After both MP and niacin treatments, serum XO activity decreased, indicating

niacin's antioxidant activity in reducing the oxidative stress that develops after I/R injury.

In this study, light microscopic examination revealed severe damage in the tissues of the ischemia group as a result of I/R injury. Ischemia developing after I/R injury has been shown to cause significant hemorrhage, edema, and necrosis. This study found damaged spinal cord areas to be infiltrated with polymorphonuclear leukocytes, lymphocytes, and plasma cells, indicating that ischemia causes an inflammatory response. The number of normal motor neurons was significantly decreased in the ischemia group, too. Histopathological evaluation of the MP and niacin groups revealed that the spinal cord was morphologically better protected from I/R injury, and a higher number of normal motor neurons were protected from injury in these groups.

The ultrastructural evaluation of the tissues in the ischemia group revealed that I/R injury caused very distinct separations in small, medium, and large myelinated axons. MP and niacin protected medium and large myelinated axons from I/R injury but unfortunately did not offer protection to small myelinated axons.

Our study evaluated neurological function using the modified Tarlov score. After the 20-minutes ischemia period, all subjects in the ischemia group were paraplegic. Both MP and niacin treatments protected the spinal cord from I/R injury and significantly improved the subjects' neurological functions.

All biochemical, histopathological, ultrastructural, and functional results of analysis in the study showed that niacin and MP offer neuroprotective effects by inhibiting apoptosis, reducing inflammation, lipid peroxidation, and oxidative stress, and preserving normal spinal cord morphology and fine structure. Both drugs also improve functionality. All effects of niacin on spinal cord I/R injury are shown in the Figure 6.

As with all experimental studies, this study has some limitations. Increasing the number of subjects in the study groups may produce more reliable results, as may more detailed grouping and different dosing regimens. This study does not examine acute

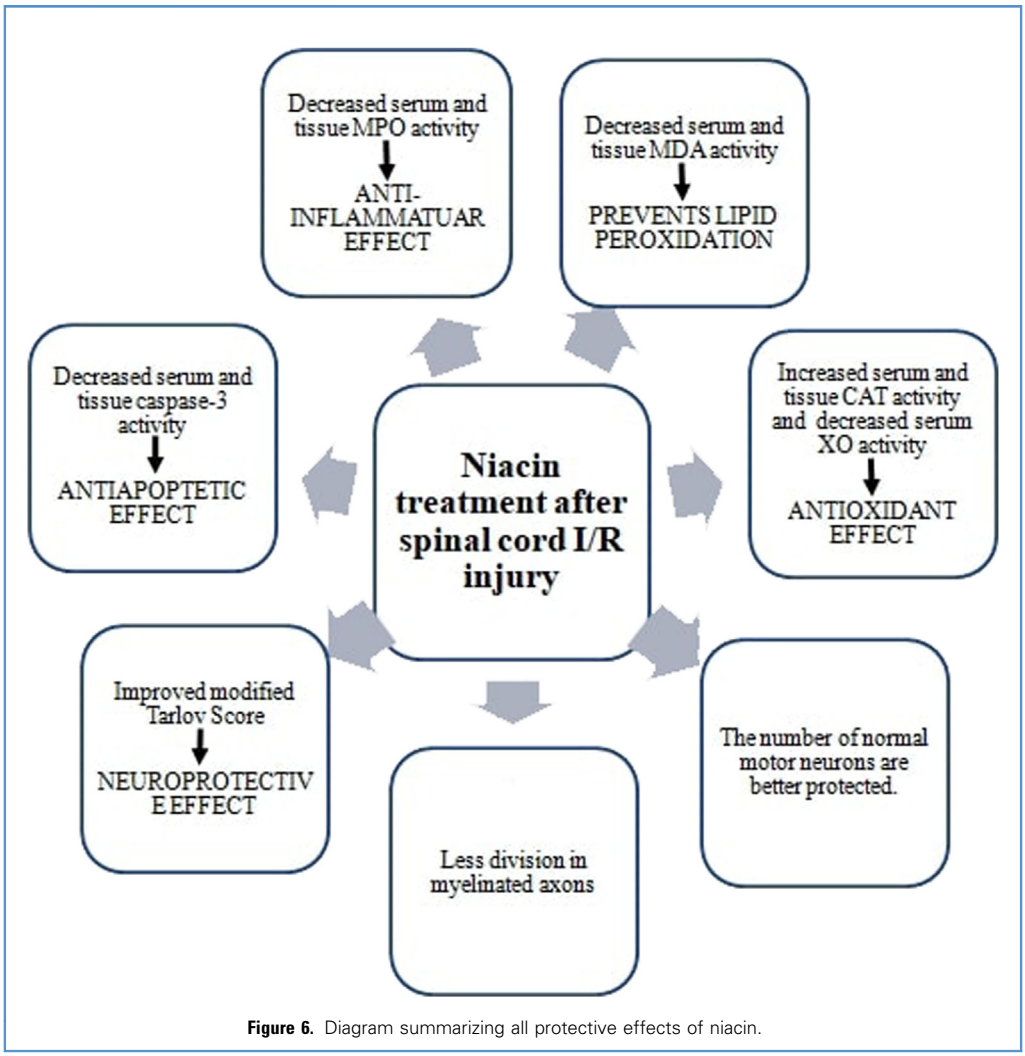

Figure 6. Diagram summarizing all protective effects of niacin.

changes in the first 24 hours following injury, limiting the potential for clinical information. In addition, niacin treatment was given to the subjects before spinal cord ischemia was produced, which is not realistic for daily practice. Further studies are required to reveal details surrounding the role of niacin in spinal cord I/R injury.

CONCLUSION

This study represents a pioneering exploration into the effects of niacin on spinal cord I/R injury, demonstrating its antiapoptotic, anti-inflammatory, and antioxidant properties that contribute to its neuroprotective role. In addition, biochemical, histopathological, ultrastructural, and functional analyses have shown that niacin provides healing in ischemic tissue after I/R injury. Our study's data, analysis, results, and conclusions show that the neuroprotective activity of niacin is at least as strong as MP. In showing niacin to be effective in protecting the spinal cord from I/R injury, we believe our study will shed light on further studies and may find clinical use.

CRediT AUTHORSHIP CONTRIBUTION STATEMENT

İlçim Ermutlu: Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Writing — review & editing, Visualization, Supervision, Project administration. **Ramazan Fesli:** Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Writing — review & editing, Visualization, Supervision, Project administration. **Ata Türker Arıkök:** Conceptualization, Methodology, Formal analysis, Investigation, Writing — review & editing, Visualization, Supervision, Project administration. **Berrin İmge Ergüder:** Conceptualization, Methodology, Formal analysis, Investigation, Writing — review & editing, Visualization, Supervision, Project administration. **Hayri Kertmen:** Conceptualization, Methodology, Formal analysis, Investigation, Writing — review & editing, Visualization, Supervision, Project administration. **Bora Gürer:** Conceptualization, Methodology, Formal analysis, Investigation, Writing — original draft, Writing — review & editing, Visualization, Supervision, Project administration.

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