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The Effects of miR-30a and miR-17 Biomarkers and Methylprednisolone in Experimental Spinal Cord Injury in Rats

Sıçanlarda Deneysel Omurilik Yaralanmasında miR-30a ve miR-17 Biyomarkerları ve Metilprednizolonun Etkileri

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Abstract

Introduction: Methylprednisolone, is a neuroprotective steroid with many effective mechanisms such as inflammation, cell blood flow changes, and apoptosis in the early period following spinal cord injury. This study aimed to demonstrate the inhibitory activity of methylprednisolone to prevent early injury through microRNA expressions, which are predicted to play a role in genomic regulation.
Method: This present study was conducted on 56 male Sprague-Dawley rats. All the animals divided into 8 groups which consists of 7 animals each. Laminectomy procedure was performed between levels T5-8. All the groups except the two control groups have been damaged with the Yasargil aneurysm clip for 1 minute at the T5 level. T5-8 spinal cord tissue was removed at the 6th, 12th, and 24th hours after clipping. Methylprednisolone was given to the intraperitoneal cavity only to the clipped groups. As a result of histopathological and immunohistochemical examination, there was a significant decrease in cell necrosis, edema, hemorrhage and white matter-gray matter transition in groups given methylprednisolone. Damaged spinal cord samples excised from all rats. miR-30a and miR-17 gene expression levels were evaluated by quantative PCR method.
Results: miR-30a was significantly upregulated at 12th and 24th hours after spinal cord injury and this rise was restricted in the methylprednisolone treated groups.. miR-17 was down-regulated at the 6th hour and and reached its lowest level at the 12th hour.
Conclusion: Methylprednisolone has statistically significant healing effects on spinal cord injury through the mechanism of miR-30a and miR-17.

Keywords: miRNA, Methylprednisolone, Spinal Cord Injury, miR-30a, miR-17.

Özet

Giriş: Metilprednizolon, omurilik yaralanması sonrası erken dönemde inflamasyon, hücre kan akımı değişiklikleri ve apoptoz gibi birçok etkili mekanizmaya sahip nöroprotektif bir steroiddir. Bu çalışma, genomik regülasyonda rol oynadığı tahmin edilen mikroRNA ifadeleri aracılığıyla metilprednizolonun erken hasarı önlemedeki inhibitör aktivitesini göstermeyi amaçlamıştır.
Yöntem: Bu çalışma 56 adet erkek Sprague-Dawley cinsi sıçan üzerinde yapılmıştır. Tüm hayvanlar, her biri 7 hayvandan oluşan 8 gruba ayrıldı. T5-8 seviyeleri arasında laminektomi işlemi uygulandı. İki kontrol grubu dışındaki tüm gruplara T5 seviyesinden 1 dakika süreyle Yaşargil anevrizma klipsi ile hasar verildi. Klipslemeden 6, 12 ve 24. saatlerde T5-8 omurilik dokusu alındı. Sadece kliplenen gruplara intraperitoneal kaviteye metilprednizolon verildi. Histopatolojik ve immünohistokimyasal inceleme sonucunda metilprednizolon

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verilen gruplarda hücre nekrozu, ödem, kanama ve beyaz cevher-gri madde geçişinde anlamlı azalma oldu. Tüm sıçanlardan eksize edilen hasarlı omurilik örnekleri. miR-30a ve miR-17 gen ekspresyon seviyeleri kantitatif PCR yöntemi ile değerlendirildi.
Bulgular: miR-30a, omurilik yaralanmasından sonra 12. ve 24. saatlerde önemli ölçüde yukarı regüle edildi ve bu artış metilprednizolon ile tedavi edilen gruplarda sınırlıydı. miR-17, 6. saatte aşağı regüle edildi ve 12. saatte en düşük seviyesine ulaştı.
Sonuç: Metilprednisolon, miR-30a ve miR-17 mekanizması aracılığıyla omurilik yaralanmasında istatistiksel olarak anlamlı iyileştirici etkilere sahiptir.

Anahtar Kelimeler: miRNA, metilprednizolon, omurilik yaralanması, miR-30a, miR-17.

INTRODUCTION

Spinal cord injury (SCI) is a type of injury with a high rate of disability. It can lead to harm or loss of sense and motor function but also may lead to multiple organ dysfunctions. The incidence of spinal cord injury varied between 13.019-163.420 per million people (1). The earliest records of SCI are in the 5000-year-old Edwin Smith papyrus. In these records, studies on soft tissue and bone tissue injuries following spinal trauma are described as incurable disorders, and research into the physiopathology and biomechanics of their alterations in neural tissue are ongoing (2).

The first known spinal cord injury in history has been reported in British naval hero Lord Admiral Sir Horatio Nelson (1758–1805). After a sniper shell that enters the chest and spinal cord told the surgeon, "All power of motion and feeling below my chest is gone." Mr. Beatty has confirmed that it is an incurable disorder (3).

There are different reasons of SCI, including falls, motor vehicle accidents/crashes, sports-related accidents, violence, and other remaining causes of injury. Motor vehicle accidents and falls are the most common causes of injury accounting for a nearly equal ratio (4).

In recent years, various animal models have been developed to allow an understanding of the complicated biomedical mechanisms of SCI and to advance medical strategies for this situation (5). Rodents are the mostly utilized animals in SCI studies because of their availability, ease of usage, and cost-effectiveness in comparison with primates (6). A cystic gap arises in the core of the spinal cord in the rat, cat, monkey, and human SCI, , which is surrounded by a rim of anatomically protected white matter (7). Previous studies in the literature provide evidence that rat models of SCI may be used to design and evaluate the structural and functional advantages of SCI treatment strategies (8).

MicroRNAs (miRNAs), a subset of noncoding RNAs, are endogenously launched small RNA molecules of ~22 nt (nucleotide) length.They may post-transcriptionally coordinate the division of target mRNAs or only suppress their translation (9). miRNAs create almost 1% of all estimated genes in nematodes, flies, and mammals (10, 11,12, 13). miRNAs are crucial for normal development and are associated with a wide range of biological functions. (14). Abnormal expression of miRNAs is related to many human illnesses (15, 16). Extracellular miRNAs have been widely notified as potential biomarkers for a diversity of diseases and they also serve as signal molecules to mediate intercellular communications (17, 18, 19).

miRNAs have key roles in the regulation of different processes in mammals. They provide a key and strong tool in gene arrangement and thus a potential new class of therapeutic targets. miRNAs largely exhibit limited complementarity with their target mRNAs in animals however, this is still enough to coordinate varied physiological processes. It has been suggested that they repress the initiation step of the translation process, which may be

followed by mRNA degradation (20). The functions of miRNAs such as; cell death and proliferation, regulation of developmental timing, physiological condition, neuronal cell fate, cardiomyocyte differentiation and proliferation, neural proliferation signaling, down-regulated in B cell chronic lymphocyte leukemia, upregulated in B-cell lymphoma, development and function of the immune system, insulin secretions and brain morphogenesis" have been reported in various publications (20-30).

This present study aimed to create spinal cord injury in rats and determine miRNAs specific to spinal cord injury in tissue and explain the recycling mechanism of damage in patients given methylprednisolone through miRNAs and be an example to future treatment plans.

METHOD

Study Population

This study was conducted in 2016 after the approval of Istanbul University Animal Experiments Local Ethics Committee dated 16.12.2016 and numbered 2016/78, following the conditions proposed by the Helsinki Declaration and Council of Europe decisions (ETS 123; 86/609/EEC). A total of 56 Sprague-Dawley male rats (16-20 weeks, weighing ranging from 300 and 350 grams) were used. During the experiment, animals were kept under standard laboratory conditions: free access to pellet feeds and water, accommodation in a temperature-controlled room (22-25°C), and a light-dark cycle of 12:12 hours were provided.

Table 1. Group Distributions

Group 1	n=7 male	Control
Group 2	n=7 male	Control 6th hour
Group 3	n=7 male	6th hour after spinal injury
Group 4	n=7 male	6th hour after spinal injury + 30 mg/kg methylprednisolone
Group 5	n=7 male	12th hour after spinal injury
Group 6	n=7 male	12th hour after spinal injury + 30 mg/kg methylprednisolone
Group 7	n=7 male	24th hour after spinal injury
Group 8	n=7 male	24th hour after spinal injury + 30 mg/kg methylprednisolone

Surgery

The surgical part of this study was carried out in the T.C. Istanbul University Prof.Dr. Aziz Sancar the Experimental Medicine Research Institute of Experimental Animal Research and Production Laboratory. Laminectomy was performed on Group 1 between T5-8 without clipping and T5-8 spinal cord tissue was excised. Laminectomy was performed on Group 2, but the spinal cord was excised at the 6th hour of laminectomy as described previously. The remaining 6 groups were clipped with the transient standard Yasargil aneurysm clip (FE 750) at 90 g pressure horizontally extradurally clamped to the dura and spinal cord circumferentially from the T5 level for 60 seconds. Spinal cord injuries were created to ensure trauma standards. After hemostasis, the layers were closed with 3/0 prolene according to anatomy. 30 mg/kg methylprednisolone (Mustafa Nevzat Drug, Istanbul) was administered intraperitoneally after clipping in groups 4-6-8,. The same procedures were performed on groups 3 and 4 at the 6th hour after clipping. Similarly, it was performed on groups 5 and 6 at the 12th hour and groups 7 and 8 at the 24th after clipping. T5-8 spinal cord tissue was excised without any damage (Table 1).

Histopathological Examination

The pathological examination and pictures of the produced preparations were performed in the T.C. Ministry of Health Beyoglu Public Hospitals Association Istanbul Okmeydani Training and Research Hospital Pathology Clinic. Spinal cord samples of all groups were placed in 10% formaldehyde solution and evaluated by a pathology specialist, who was employed in the Pathology Clinic of Okmeydanı Training and Research Hospital, who was unaware of the treatment groups, the treatments applied and the neurological evaluation results. After fixation, the samples were embedded in paraffin blocks and 5µm thick serial slicess were taken with a microtome and stained with Hematoxylin-Eosin (H-E). The preparations were examined histologically and morphologically with a light microscope (Zeiss, Oberkochem, Germany) at x40, x100, x200, and x400 magnifications.

Immunohistochemical Examination

Immunohistochemical staining was performed in Istanbul Sisli Tuzlali Pathology & Cytology Laboratory. TNF-α, IL-1, and IL-1β Antibodies were used for immunohistochemical examination. An immunohistochemical fixation protocol was applied (31). After the sections on the glass slides were deparaffinized, they were heated in the Citrate Buffer (pH:6) solution for proteolysis, at 700 Watts, in a microwave oven for 3X 5 minutes. Tissues were then incubated in a 3% H2O2 solution to prevent endogenous peroxidase activity. Following washing with 1Xphosphate buffer solution (PBS), the sections were incubated with serum for 60 min to prevent non-specific protein binding. was treated. Then, 1:300 diluted TNF-α, IL-1, and IL-1β were dripped onto the sections and kept at +4°C for overnight. Biotinylated secondary antibody was dripped onto the sections and incubated for 15 min at room temperature. It was kept for 15 minutes at room temperature in the streptavidin-HRP complex after washing and incubation. In the last step, 3,3'-diaminobenzidine (DAP) was used as the chromogen.

miRNA Isolation

The spinal tissues (~4 cm long and 0.5 cm thick), which were acquired via laminectomy in the lab employing experimental animals, were transferred by ice block to the lab where the analyses would be done (Anotolia Geneworks (Anatolia Diagnosis and Biotechnology Products R&D Industry and Trade Co. Kadikoy/Istanbul, Turkey)) within the first two hours. MicroRNA isolation from Serum, tissue and paraffin embedded tissue samples were performed by using Magrev microRNA Extraction Kit'. By following the company's instructions e ' First of all, Tissue miRNA Homogenization Buffer was added to the tissue samples and pretreated by pipetting. After pipetting, the tissue was incubated at 56 ºC for 45-80 minutes, during the incubation, the tubes were placed back in the heater by vortexing every 10 minutes.

Add 800 µl of MiR-Buffer 1 and 20 µl of Proteinase K to the LB tubes. Tissue sample treated with 400 µl buffer was transferred to each tube and mixed with a pipette. The magnetic block was placed on the stand and waited for 2 minutes. After the liquid in the tube was pipetted away, the magnetic block was removed, and the magnetic beads were suspended with 1000 µl of MiR-Buffer 2. This mixture was transferred to 1.5 ml microcentrifuge tubes located under the Magrev stand, and the magnetic slide at the bottom was pulled forward and waited for 1 minute. The liquid in the tube was carefully removed and 1000 µl of MiR-Buffer 3 was added while the magnetic slide was pulled. After it became homogeneous with a pipette, the inside of the tubes were washed and then MiR-Buffer 3 was removed. For DNAse 1 application, the

magnetic block was removed at this stage and the beads were suspended with 100 µl of DNAse 1. To prepare the DNAse 1 mix, 5 U of DNAse 1, DNAse Reaction Buffer 1 X and dH2O were made up to 100 µl in total volume. The mixture was incubated for 10 minutes at 37 °C.

After the tubes were removed from the heater block, 200 µl of MiR-Buffer 1 was added, and they were left at room temperature for 5 minutes. The samples were taken to the unit at the bottom, and after the sled was pulled forward, they were waited for 1 minute and the liquid in the tube was removed. Afterwards, the magnetic beads were suspended by adding 500 µl of MiR-Buffer 2 and the sled was pulled forward and waited for 1 minute. The liquid in the tube was removed and suspended by adding 500 µl of MiR-Buffer 4, the slide was pulled forward for 1 minute and the liquid was removed. MiR-Buffer 4 application was applied once again in the same way. While the magnetic slide was pulled, 1000 µl of MiR-Buffer 3 was added to the magnetic beads and the inside of the tubes were washed with this buffer, and then the buffer was removed. Afterwards, the tubes were treated with MiR-Buffer 6 with a volume of 500 µl, and the liquid was removed 1 minute after the sled was pulled. After the magnetic slide was pushed into place, 60 µl of MiR-Buffer 5 was added to the tubes and the tubes were incubated at 95 ºC for 10 minutes. After incubation, the tubes were placed in the lower unit and the sled was pulled forward and waited for 1 minute. The resulting elution was transferred to a clean tube and stored at -20 ºC for long-term storage.

Insulation Efficiency Analysis

Spectrophotometric Analysis

Spectrophotometric measurements of isolated miRNAs were performed by taking 1 µl from each sample (NanoPhotometer P 300, IMPLEN). Elution buffer (Buffer-5), which was used during Total RNA isolation, was used as the 'Blank'. The purity values of the RNAs isolated from the serum were 47.80 -118.00 ng/µl and their concentrations were determined. A 260/280 ratios were read between 1.84-2.01. Each RNA sample was diluted to 200 ng/µl to enter the PCR according to its concentration (Table 2).

Real Time PCR Analysis

rno-miR-24-3p and 2 different miRNAs (rno-miR-17-5p and rno-miR-30a-5p) were studied for normalization for a control 7 groups. The reaction was studied in 200 µl thin-walled PCR tubes with a 50 µl volume of Montania 4896 Real Time PCR Device.

Table 2. miRNA Primers (58)

miRNA Primers		
Primers	**(5‘-3‘)**	**Target Gene**
Primer 1-F	TGTAAACATCCTCGACTGGAAG	rno-miR-30a-5p
Primer 2-F	GGCAAAGTGCTTACAGTGC	rno-miR-17-5p
Primer 3-F	GTTTGGCTCAGTTCAGCAG	rno-miR-24-3p
Primer 4-R	GTGCAGGGTCCGAGGT	Universal R
Primer 5-F	GGGTGTAAACATCCTCGAC	rno-miR-30a-5p (not worked)
Primer 6-F	TGTGTTGTGTAAACATCCTCGAC	rno-miR-30a-5p (low yield)

PerfeCTa Universal PCR Primer (Quanta Biosciences) was used with miRNA forward primers. In addition, 2 different forward primers were used for miRNA 30a-5p, but

amplification data could not be obtained from 5-F, and amplification results with low efficiency (at high Ct) were obtained from 6-F.

In addition, a second Dnase 1 (Quanta Biosciences) application was performed to remove genomic DNA from the isolates. For this purpose, 1 µl of 10 x Reaction Buffer and 2 µl of PerfeCTa Dnase 1 for 7 µl of RNA were mixed by vortexing. After the mixture was incubated at 37 ºC for 30 minutes in PCR tubes, 1 µl of Stop Buffer was added and Dnase 1 activity was inactivated at 65º C for 10 minutes.

MiR-17, miR-24 and miR-30a were screened and their use as biomarkers were analyzed in the isolated control and experimental groups. In this context, qScript microRNA cDNA Synthesis Kit (Quanta Biosciences) was used to convert miRNAs to cDNA.

First of all, for the poly A tail addition reaction a mix is created and poly A tail synthesis incubations are performed at 37º C for 60 minutes and at 70º C for 5 minutes (Table 3).

Table 3. Poly A Tail Addition Reaction

Reaction Components	Concentration	Used Volume (µl)
Poly (A) Tailing Buffer	5X	2
Poly (A) Polymerase		1
Total RNA	100ng- 1 ug	7
	Total Volume	10 µl

For cDNA synthesis a mixture is created and incubated at 42 ºC for 20 minutes and at 85ºC for 5 minutes. PerfeCTa SYBR Green SuperMix (Quanta Biosciences) was used as a mix for Real Time PCR amplification of miRNAs translated into cDNA (Table 4).

Table 4. cDNA Synthesis

Reaction Components	Concentration	Used Volume (µl)
Poly A Reaction		10
MicroRNA cDNA Reaction Mix		9
qScript Reverse Transkriptase		1
	Total Volume	20 µl

For the amplification of miRNAs with Real Time PCR, a final volume of 50 µl is created in total and the amplification is performed with the following thermal protocol (Table 5) (Table 6).

Table 5. Amplifications With Real Time PCR

Reaction Components	Concentration	Used Volume (µl)
PerfeCTa SYBR Green SuperMix	2X	25
PerfeCTa microRNA Assay Primer (miR-127 ve miR-21)	10 µM	1
PerfeCTa Universal PCR Primer	10 µM	1
MicroRNA cDNA	(1-10 ng total RNA)	23
	Total Volume	50 µl

Ct (cycle threshold) is the name given to the number of cycles (threshold cycle) in real time PCR experiments where the amount of fluorescent signal exceeds the minimum value (threshold value) required to be observed. Ct parameter; Indicates the number of cycles in

which the detected fluorescence threshold is exceeded. Increases in PCR are logarithmic, so the following points should be taken into account when calculating the fold difference.

Table 6. Thermal Protocol for Amplification

Taq Polimerase activation	**95 ºC**	**2 minute**	
PCR Cycle	95 ºC	5 seconds	40 Cycle
	60 ºC	15 seconds	
	70 ºC	15 seconds	
	(floresan veri toplama)		
Melting Curve Analyse	50 ºC -90 ºC		

The 2-ΔΔCT method has been widely used as a relative quantification strategy for quantitative real-time polymerase chain reaction (qPCR) data analysis (32).

Values in a thermal protocol; It represents bindings between 0 and 40, and values close to zero indicate higher values. Values close to forty indicate a lower value.

The difference between the values in the thermal protocol is converted into a mathematical expression with 2-ΔΔCT (-ΔΔCT = amount of change) (change value over 2), followed by the expression increased if the value is displaced towards zero, and decreased if it is displaced towards forty.

Statistical Analysis

SPSS Windows version 24.0 package program was used for statistical analysis and $P<0.05$ was accepted as statistically significant. The conformity of the data to the normal distribution was tested with the Shaphiro Wilk test. In the comparison of numerical data in more than 2 independent groups, one-way analysis of variance (ANOVA) and LSD multiple comparison tests were used for normally distributed features, and Kruskal Wallis test and All pairwise multiple comparison test were used for non-normally distributed features. As descriptive statistics, mean±standard deviation was given for numerical variables, and number and % values were given for categorical variables.

RESULTS

When real time PCR results are examined;

⇒ According to the ΔΔCt results, the miR-30a was upregulated.

⇒ miR-17 was down regulated (Figure 1)

As a result of the statistical analysis;

- According to Kruskall Wallis test results for both miR-30a and miR-17, statistical difference between the groups was determined.

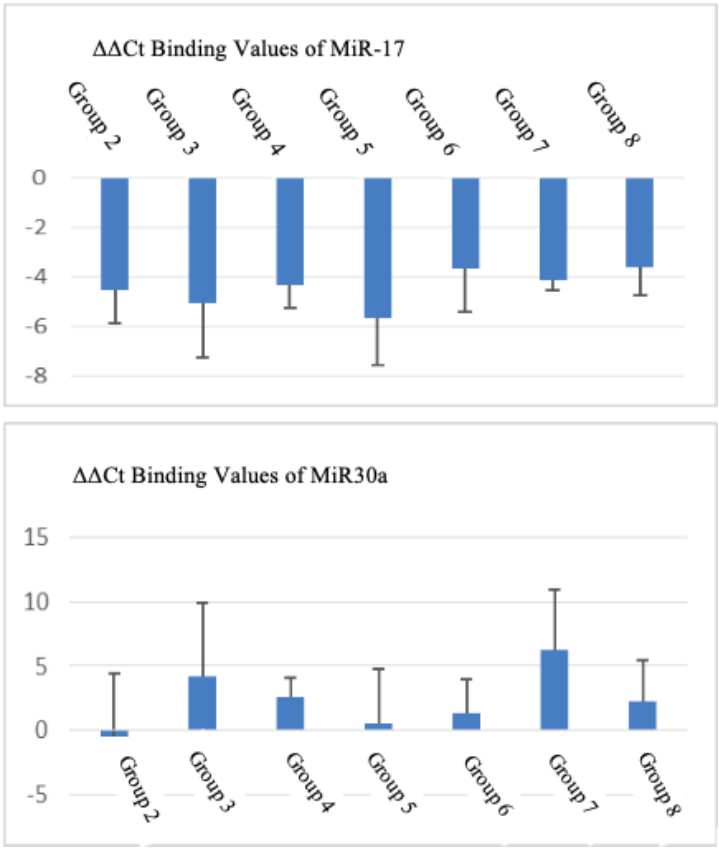

Figure 1. ΔΔCt Binding Values of MiR-17 and MiR30a.

All pairwise test was used for the difference between groups. According to the results of this test;

There was a significant difference of :

- miR-30a levels, between the 6th hour after laminectomy (Group 2) and the 24th hour after the damage (Group 7).
- miR-30a levels, between the 12th hour (Group 5) and the 24th hour (Group 7).
- miR-30a levels, between the 12th hour with methylprednisolone (Group 6) and the 24th hour without methylprednisolone (Group 7).
- miR-17 levels, between the 6th hour (Group 3) and the 24th hour with methylprednisolone (Group 8).
- miR-17 levels, between the 12th hour (Group 5) and the 12th hour with methylprednisolone (Group 6).
- miR-17 levels, between the 12th hour (Group 5) and the 24th hour (Group 7) .
- miR-17 levels, a between the 12th hour (Group 5) and the 24th hour with methylprednisolone (Group 8).

The gray matter-white matter transition was regular, the structures of neurons were in normal morphology and protected. No edematous tissues or necrosis was observed in the sections in the samples belonging to the control group (Figure 2).

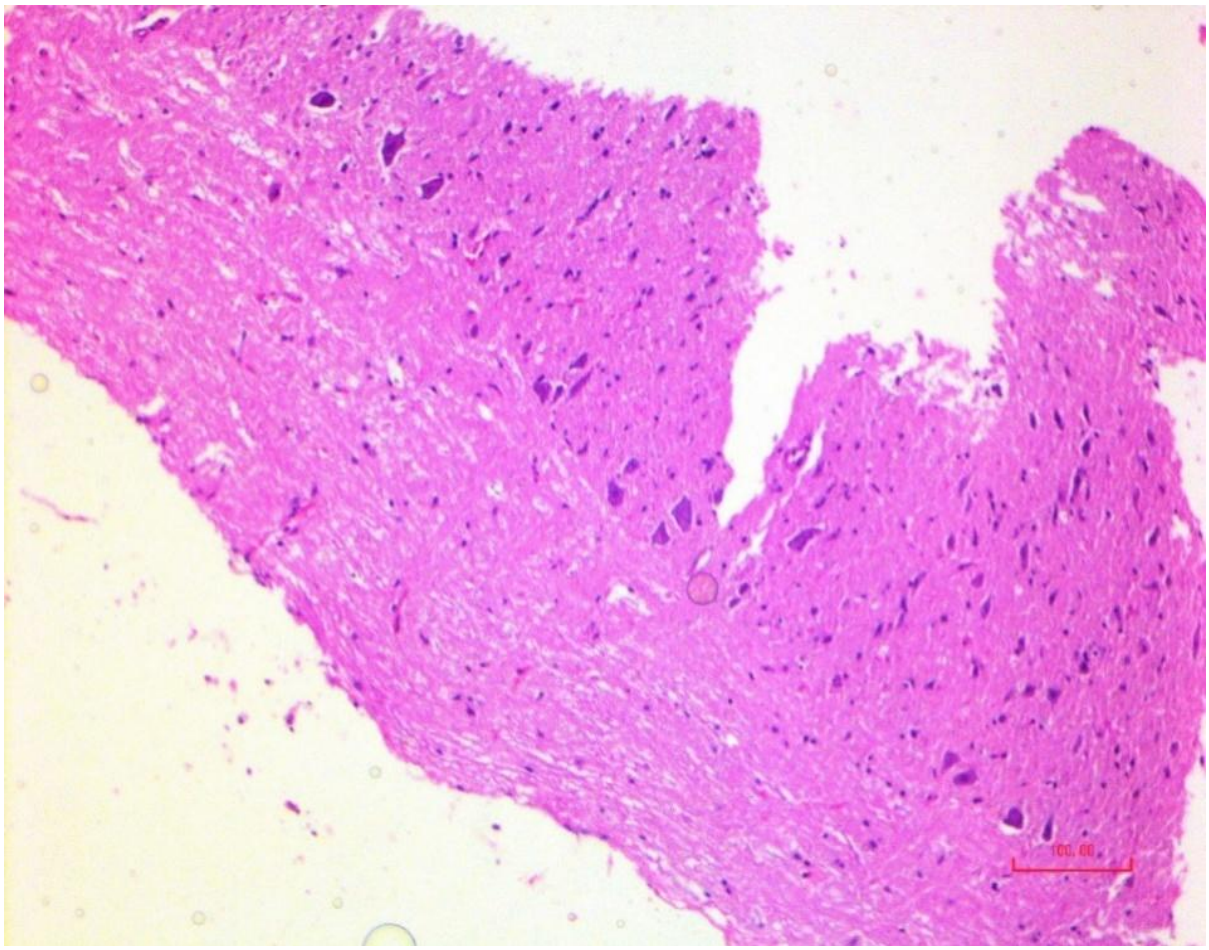

Figure 2. Examination of the control group with Haematoxylin-Eosin dye at x10 magnification. Gray matter-white matter transition is regular. Neurons in normal morphology.

The account of neurons decreased partially in the samples taken at the 6th hour (Group 3). Focal neuronal apoptosis was detected in some areas. The gray-white matter transition was regular. Mild focal edema were identified (Figure 3a). Focal light staining was observed in TNF-α, IL1, and IL1β staining. The account of neurons increased in the 6th-hour samples with methylprednisolone (Group 4) compared to the group without (Group 3). Apoptotic neurons and edema were decreased (Figure 3b). The cytoplasm dimension was decreased compared to nucleus in the samples taken at the 12th hour after spinal damage. The gray-white matter transition was vanished. Widespread apoptosis, hemorrhagic areas and mild edema were observed (Figure 3c). The neurons in regular morphology were partially increased at the 12th hour with methylprednisolone (Group 6) compared to the 12th hour without (Group 5). The gray matter-white matter transition was slightly fainted (Figure 3d). Hemorrhage was not observed in samples taken 24 hour (Group 7). Nissl bodies were detected in the cytoplasm. A large number of apoptotic dense nuclei neurons was observed (Figure 3e). Normal-looking neurons were significantly increased at the 24th hour with methylprednisolone (Group 8). Nissl bodies were decreased compared to those without methylprednisolone (Group 7). The gray matter-white matter transition became evident (Figure 3f).

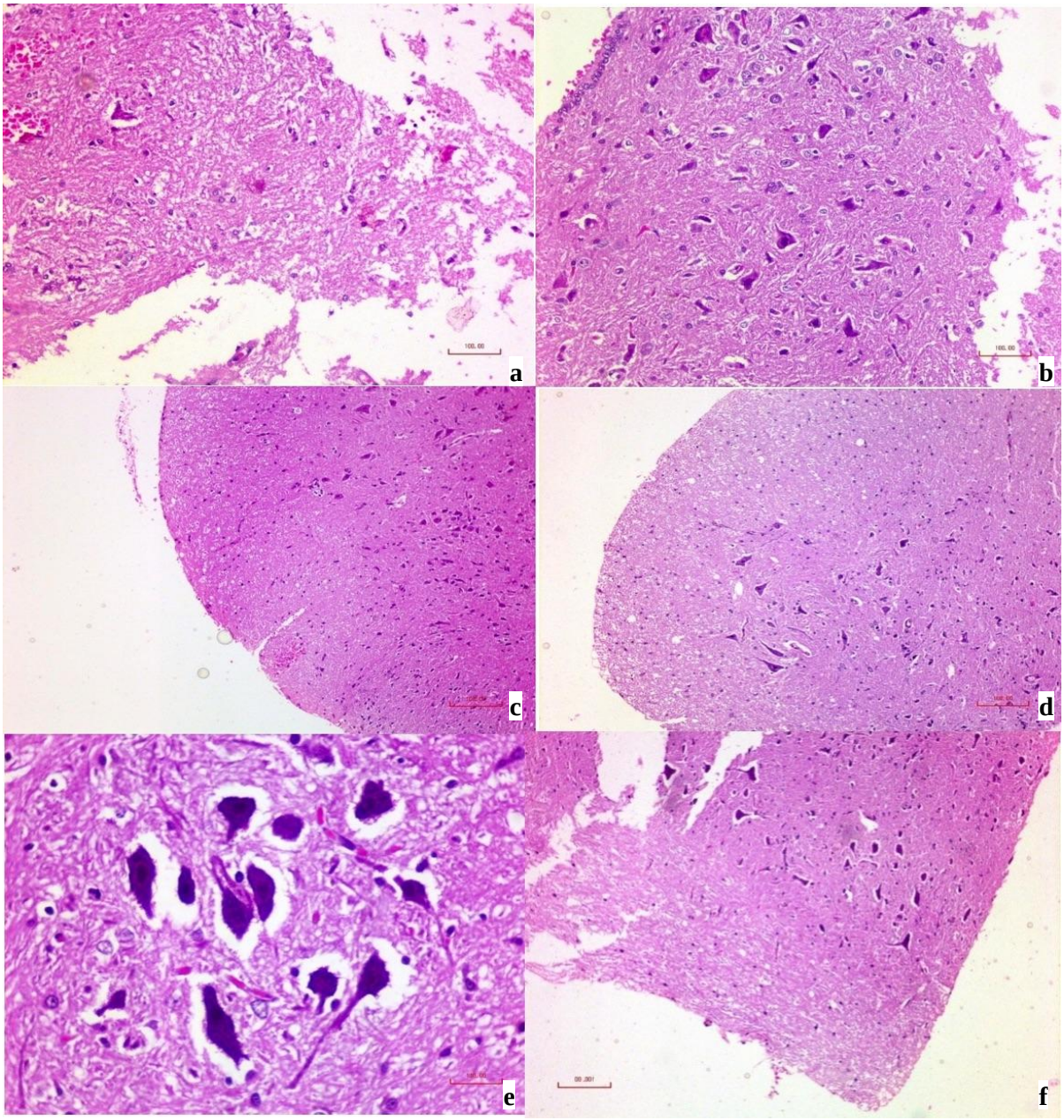

Figure 3. a) Image of the tissue at x20 magnification with Hematoxylin-Eosin dye at 6th hour after spinal damage. It appears that the number of neurons is reduced and there is mild edema. b) Examination of the group given methylprednisolone at the 6th hour after spinal injury with Hematoxylin-Eosin dye by x20 magnification. It was observed that the number of neurons increased compared to the group with spinal damage. c) Investigation of the group at the 12th hour after spinal injury with Hematoxylin-Eosin dye at x10 magnification. Significant reduction in gray-white matter transition was observed. d) Examination of the group given methylprednisolone at the 12th hour after spinal injury with Hematoxylin-Eosin dye by x10 magnification. It was observed that neurons in regular morphology increased. e) Examination of the group at the 24th hour after spinal injury with Hematoxylin-Eosin dye by x40 magnification. Nissl bodies showing. f) Examination of the group given methylprednisolone at the 24th hour after spinal injury with Hematoxylin-Eosin dye by x10 magnification. It was observed that the transition of gray-white matter became evident.

DISCUSSION

The majority of those exposed to spinal cord injuries are young active adults. For this reason, it is of great importance to investigate the methods of treatment both personally and communally and to conduct studies in this regard especially for the morbidity of the patients.

In addition to surgical and physical therapy combinations, pharmacological research continues throughout the world for spinal injuries. In these studies, it is mainly aimed, to prevent apoptosis by reducing the secondary effect of spinal cord injury, to increase axon regeneration, to prevent neuronal tissue replacement, scar and gliosis formation.

The effects of steroids have been emphasized especially in spinal cord injuries in the last century. For this purpose, many effects of steroids on the spinal cord have been investigated. Steroids have been shown to specifically prevent potassium loss from damaged cord tissue and facilitate extracellular calcium reuptake (33). The most beneficial effects of steroids in spinal cord injury are their inhibitory effects on lipid peroxidation and the maintenance of metabolic functions. It is also effective in reducing posttraumatic ischemia and slowing traumatic ion exchange (34).

Due to the widespread use of methylprednisolone in spinal cord injury, the NASCIS III study was initiated to investigate the effect of TrilazadmesylateTM treatment, which acts with the same mechanism due to its side effects. According to the results of this study, it was found that there was no difference between the patients when Trilazadmesylate and methylprednisolone were given in the first 3 hours, and methylprednisolone treatment has been found to be more effective in correcting motor functions between 3-8 hours (35).

Currently, although the effect and side effect profile of methylprednisolone is discussed, it has been proven to be effective on the lipid peroxidation mechanism. As a result of recent studies of human genes and functions, it has been revealed that the genome transcribes thousands of regulatory "non-coding" RNA (ncRNA) involved in live metabolism and reactions. These include micro RNA (miRNA), small interfering RNA (siRNA), P-element-induced Wimply Test (PIWI), interacting RNAs (piRNA), and various "long non-coding RNA" (ncRNA) (36).

miRNA biogenesis begins with the processing of RNA polymerase II-RNA polymerase III transcripts post or co transcriptionally (37). Approximately half of all now defined miRNAs are intragenic and processed usually from introns and comparatively few exons of protein coding genes, while the remaining are intergenic, transcribed independently of a host gene and arranged by their its promoters (38,39). Occasionally miRNAs are transcribed as one lengthy transcript named clusters, which may have similar seed regions, and in this status they are considered a family (40). The biogenesis of miRNA is categorized into canonical and non-canonical pathways (41).

Most researches until today have been showed that miRNAs bind to a specific sequence at the 3′-UTR of their target mRNAs to induce translational repression and mRNA deacetylation and decapping (42, 43). miRNA binding places have also been detected in other mRNA regions including the 5′-UTR and coding sequence, as well as within promoter regions (43). The linking of miRNAs to 5′-UTR and coding regions have silencing effects on gene expression while miRNA interaction with promoter region has been declared to induce transcription (44, 45). On the other hand, more studies are required to fully understand the functional importance of this kind of interplay (41).

Studies have been conducted on the relationship of microRNAs to many neuronal diseases and neurodegeneration, and the examination of the changes in spinal cord injury have been recently (46, 47). MiRNA values changed after spinal cord injury in rats were started to have been investigated by microarray analysis and real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) method. Previous studies have been shown increases in miRNA in spinal cord tissue (48).

Afterwards, was detected 269 of 350 miRNAs that were studied as a result of microarray analysis in the spinal cord of adult rats by Liu et al. These miRNAs were divided into four groups according to signal changes, those with low level (intensity <500), medium level (500–4999), high level (5000–9999) and highest level (>1000). In this study, significant changes was observed in 60 of 269 miRNAs detected after spinal cord injury. Changes in MiRNAs appeared at different times as down and up-regulation. Among these, it was observed that the miR-17 we selected in our study was down-regulated in the first 4 hours after spinal cord injury and showed marked upregulation on the 1st and 7th days. They also mentioned that miR17 along with miR-21, miR-145, miR-214, miR-133a, miR-133b, miR-674-5p, miR-15b, miR-20a, miR206, miR-672, miR-103 and miR-107, is a potential target for anti-apoptotic gene Bcl2-1 and Bcl2-2 after spinal cord injury of these miRNAs (49).

In another study under the action of intercellular adhesion molecule 1 (ICAM1), Interleukin-1β (IL-1β) and Tumor Necrosis Factor-α (TNF-α) miR-181a, miR-411, miR-99a, miR-34a MiRNAs such as miR-30c, miR-384-5p and miR-30b-5p, miR-486 have been found to be downregulated, while miR-17, miR-20 and miR-124a are upregulated (50-53).

MiR-17 has been shown to take part in the inflammatory process that results in spinal cord injury. Upon the start of the inflammatory process, of miR-17 was held responsible for upregulation on the 1th day. In our study, the first 24-hour early spinal injury period was studied. As a result of our study, it was observed that miRNA-17-5p, which is a member of the miR-17 family, is the most downregulated in the 12th hour and this downregulation decreases significantly in the 24th hour. It was found that this downregulation, which occurred at the 12th hour, was significantly reduced with methylprednisolone. As a result of these results, it was determined that miR-17-5p was downregulated in the first 24 hours to peak at the 12th hour of spinal cord damage and that miR-17 down-regulation decreased at the 12th hour by administration of methylprednisolone. It was previously known that MiR-17-5p was upregulated after at the 24th hour for proliferation of cells such as astrocytes and oligodendrocytes in spinal cord injury. In our study, it is thought that miR-17-5p, which was detected to be down-regulated at the 12th hour, may also play a role in decreasing cell proliferation at the 24th hour. It was also stated in another study that miR-17 is specific for oligodendrocytes and increases the increase of oligodendrocyte proliferation cells by inhibition of the AKT pathway (54).

In a study by Chang et al., miR-30a was shown to be down-regulated. In our study, we determined that the spinal cord injury of miR-30a-5p was upregulated in the first 24 hours, which is the early damage period (55). It was observed that miR-30a-5p has the highest upregulation at the 6th hour after spinal injury, then this increase decreases and at the end of the 24th hour, the upregulation was at the lowest level. It was understood that the methylprednisolone is not effective at the 12th hour, by looking at the values of the 12th hour of the group with and without methylprednisolone. However, at the end of 24th hour, methylprednisolone was detected a decrease in miR-30a-5p upregulation. As can be seen from this, it is thought that methylprednisolone reduces miR-30a upregulation by affecting damage mechanisms occurring at the 24th hour.

Important steps have been taken with genetic studies on the mechanism of spinal cord injury. MicroRNAs has been shown to play an effective role in damage mechanisms, and methylprednisolone has been shown to be effective in providing treatment by affecting these microRNAs (56, 57). In the early injury period after spinal cord trauma, miRNAs previously detected in the spinal cord were shown to be up-regulated and down-regulated. Changes in this microRNA have been shown to vary with methylprednisolone, which is used in spinal cord injury.

CONCLUSION

In this study, it was observed that miR-17-5p was down-regulated and miR30a-5p was up-regulated after spinal cord injury in male Sprague-Dawley rats. In addition, the effect of methylprednisolone, which has been proven to have an effect on spinal cord damage but is controversial due to its side-effect profile, was investigated. It has been determined that miR-17-5p is effective in the down-regulation at the 12th hour after spinal cord injury with the effect of methylprednisolone. It was determined that the up-regulation of MiR-30a-5p was significantly reduced at the 24th hour by the effect of methylprednisolone.

It has been shown that miRNAs previously detected in the spinal cord are up-regulated and down-regulated in the early injury period after spinal cord trauma. It has been shown that changes in this microRNA change with methylprednisolone, which is used in spinal cord injury. Considering the side-effect profile of methylprednisolone, this study will lead to the development of new treatment methods over these microRNAs in the coming years, by revealing the mechanisms of microRNAs that can act on the spinal cord.

This study will lead to the development of new treatment methods over these microRNAs in the coming years by revealing the mechanisms of microRNAs that may have an effect on the spinal cord, considering the side effect profile of methylprednisolone.

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Ethical Declaration: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Ethics committee approval has been granted from our institution. As this was a retrospective research no informed consent has been obtained from participants.

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