

## **The therapeutic effects of cyclosporin-A on experimental spinal cord injury.**

**Ali Riza Gezici<sup>1</sup>, Guven Kilic<sup>1</sup>, Tulin Firat<sup>2</sup>, Seckin Emre Cancan<sup>1\*</sup>, Aysel Kukner<sup>2</sup>, Nezh Ozkan<sup>1</sup>, Yasar Dagistan<sup>1</sup>**

<sup>1</sup>Department of Neurosurgery, School of Medicine, Abant Izzet Baysal University, Bolu-Turkey

<sup>2</sup>Department of Histology and Embryology, School of Medicine, Abant Izzet Baysal University, Bolu-Turkey

### **Abstract**

**Background:** According to the experiments, neutrophils and microglial cells are the first to attend the early phase of events in inflammatory response to SCI. Those pilot cells are seen in the first 12-24 hours and disappear about 3-5 days. The neutrophil accumulation and activation are steered by many cytokines such as TNF- $\alpha$ , IL-1 and IL-6. Neutrophils do accompany to the modulation of secondary injury mechanisms via neutrophil proteases and reactive oxygen molecules. When those processes are taken into account, depletion of neutrophils or depression of their functions may derive neuro-protection and neurological healing.

**Purpose:** To investigate the therapeutic and neuroprotective effects of Cyclosporin-A (CSA) on recovery processes using clinical and histopathological tests, which has not been used very frequently in clip compression spinal cord injury (SCI) models.

**Material and methods:** Twenty-four Sprague-Dawley rats were divided into three groups: group 1 [Sham-control, n=8], group 2 [SCI+2 mL saline intramuscular (i.m.), n=8], group 3 [SCI+5 mg/kg CSA (i.p.) 1 h after SCI and for the following three days, n=8]. Rats were evaluated 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 10<sup>th</sup> days after SCI, clinically by Drummond and Moore scale and under light microscopy and by TUNEL test; after scarification on 10<sup>th</sup> day.

**Results:** Clinical and histopathological results of treatment group were found significantly better than the results of the trauma group.

**Conclusion:** CSA can depress apoptosis and necrosis rates in a statistically significant manner and carry out the statistical difference in clinical results.

**Keywords:** Animals, Cyclosporine, Immunosuppressive agents, Motor activity, Spinal cord injuries, Thoracic vertebrae.

*Accepted on February 03, 2017*

### **Introduction**

First reaction of body to injury and infections is particularly the inflammation. The role of inflammation after spinal cord injury (SCI) is defined in details; but profitable and destructive effects are still debatable. In general aspect, although early stage inflammatory events are not welcomed in neurotrauma, they are thought to be in favour on late stages [1]. According to the experiments on animals and humans, neutrophils and microglial cells are the first to attend the early phase of events in inflammatory response to SCI [2-5]. Those pilot cells are seen in the first 12-24 hours and disappear about in 3-5 days [1]. The neutrophil accumulation and activation are steered by many cytokines such as TNF- $\alpha$ , IL-1 and IL-6 [6]. Neutrophils do accompany to the modulation of secondary injury mechanisms via neutrophil proteases and reactive oxygen molecules [7]. Minutes or even hours after SCI, those cells are activated or transform into macrophages. Macrophages add more to the destructive effects by releasing pro-inflammatory cytokines, reactive oxygen radicals, nitrous oxide and

proteases [8]. They lead many biological substrates to change in a pathological manner, such as peroxidation of the lipid components of the oxidative stress cells. The results of an early staged inflammation; like ischemia, cell/tissue edema, oxidative degradation, myelin degradation, necrosis and apoptotic changes, may increase the volume of the lesion [9]. Furthermore, those changes give rise to glial scar tissue and development of the infection protective environment; thence hinder creation of a successful regeneration [10]. When those processes are taken into account, depletion of neutrophils or depression of their functions may derive neuro-protection and neurological healing [11].

This study aims to investigate the protective (neuroprotective) and therapeutic (regenerative) effects of the cyclosporin-A, which is an immunosuppressive drug, on secondary spinal cord injury in a rat spinal injury model; in structural and also functional aspects.

## Material and Methods

### Experimental groups

The investigation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication no. 85-23, revised 1996) and approval was received from the Institutional Animal Ethics Committee at Abant İzzet Baysal University. Twenty four female and adult Sprague Dawley rats, weighing 250-300 g, were divided randomly into three groups; composed of eight rats in each:

Group 1: Control-Sham (only laminectomy, no SCI, n=8)

Group 2: Trauma (SCI, 2 mL saline intraperitoneally [i.p], n=8)

Group 3: Trauma+CSA (SCI, single 5 mg/kg cyclosporin-A [Sandimmun Novartis] injected i.p. immediately after SCI and for the following three days, once a day, n=8)

All rats were evaluated on 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 10<sup>th</sup> days after SCI, clinically by Drummond and Moore scale, under light microscopy and by TUNEL test; after sacrifice on day 10.

### Surgical procedure

The rats were anesthetized with intramuscular injection of 10 mg/kg xylazine (Bayer, Istanbul, Turkey) and 60 mg/kg ketamine hydrochloride (Parke Davis, Istanbul, Turkey) before surgery. With the rats in a prone position, a T6-T10 midline skin incision was made. Three-level laminectomies (T7-T9) were performed, leaving the dura matter intact, and SCI was produced by extradural compression of the spinal cord using an aneurysm clip with a closing force of 24 g. In all of the injured groups, the spinal cord was compressed for 1 min. Sham-injured animals were only subjected to laminectomy. After surgery, 2.0 cc of saline was administered intraperitoneally to replace the blood volume lost during the surgery. During recovery from anaesthesia, the rats were placed on a warm heating pad and covered with a warm towel. The rats were singly housed in a temperature-controlled room at 27°C for a survival period of 10 days. Food and water were provided to the rat ad libitum. During this time period, the bladders of the animals were manually voided twice a day until the mice were able to regain normal bladder function. At the end of day 10, all rats were killed under deep anaesthesia.

Approximately 10 mm of spinal cord from the level between T7 and T9 was obtained from each rat, and in the trauma groups the cord sample was divided 3 mm below the epicenter of the injury. The samples from the lower levels of the spinal cord lesion (epicenter) were used for histological and immunohistological analyses.

### Neurological evaluation

**Grading of motor disturbance:** The hind limb motor function of rats were evaluated once a day on days 1, 3, 5 and 10 after SCI by an independent observer according to the Drummond

and Moore scale [12]. A score of 0 to 4 was assigned to each animal as follows: 0=paraplegic with no evident lower extremity motor function; 1=poor lower extremity motor function, flicker of movement, weak antigravity movement only; 2=moderate lower extremity function with good antigravity strength but inability to draw legs under body and/or hop; 3=the ability to draw legs under body and hop, but not normally; 4=normal motor function.

### Histological evaluation

**Light microscopy:** Spinal cord biopsies were taken on 10<sup>th</sup> day. The biopsies were fixed for 24 h in paraformaldehyde solution (4% in 0.1 M PBS) at room temperature, dehydrated by graded ethanol, and embedded in paraplast (Sherwood Medical, Mahwah, NJ). Tissue sections (thickness 5\_μm) were deparaffinised with xylene, stained with H&E and studied using light microscopy (Dialux 22 Leitz; DBA srl). All the histological studies were performed in a blinded fashion. Extensity of the necrosis was evaluated in three main specimens which were collected 3 mm caudal to the epicenter and specimens were analyzed for 4 criteria established for acute spinal cord injury by Black et al. [13], as follows: 1-white matter degeneration, characterized by edema, formation of cysts, demyelination and infiltration of macrophages, cystic necrosis and cytoarchitectonic disorganization. 2-hemorrhage in white or gray matter; 3-neuronal loss, sometimes with vacuolization and inflammatory infiltration in gray matter; 4-signs of hypoxic injury: nuclear retraction and pyknosis, as well as intense eosinophilic staining of the pericardium. Based on the described criteria, histological alterations related to the intensity of necrosis were classified semi-quantitatively into the following four categories after scanning of all slices; <1% of total scanned area: score 0; 1-24% of total area: score 1; 25-49%: score 2; 50-74%: 3; and finally >75%: score 4. This quantification was performed by one independent and experienced pathologist on three different sections in a blinded manner and without knowledge of the experimental group.

### Terminal Deoxynucleotidyltransferase-Mediated UTP end labelling assay (TUNEL)

TUNEL assay was conducted by using a TUNEL detection kit according to the manufacturer's instruction (Apotag, HRP kit; DBA srl). In brief, sections were incubated with 15 g/ml proteinase K for 15 min at room temperature and then washed with PBS. Endogenous peroxidase was inactivated by 3% H<sub>2</sub>O<sub>2</sub> for 5 min at room temperature and then washed with PBS. Sections were immersed in TdT buffer containing deoxynucleotidyl transferase and biotinylated UTP in TdT buffer, incubated in a humid atmosphere at 37°C for 90 min, and then washed with PBS. The sections were incubated at room temperature for 30 min with anti-horseradish peroxidase-conjugated antibody, and the signals were visualized with diaminobenzidine. The site of the trauma on the spinal cord was accepted as the "epicenter" and apoptotic cells in three different main sections which were collected 3mm caudal to the epicenter, were counted.

**Statistical analysis**

SPSS (Statistical Package for Social Sciences) for Windows 10.0 was used in the analysis. Since parameters had no regular distribution, Kruskal Wallis test was used in comparisons of quantitative data from groups. And Mann-Whitney U test was preferred to detect the group which causes the difference. When comparing the parameters within the groups, Wilcoxon signed rank test was used. Results were evaluated in a 95% confidence interval and the significance was accepted at the level of  $p < 0.05$ .

**Results**

Reduction in the body weights of the subjects before and after the experiment was significant. After day 7, significant atrophy in the lower extremity muscles was observed in the trauma group whereas it was not prominent in the treatment group. After the sacrifice of the subjects, macroscopic edema and hemorrhage was seen on extracted medulla spinalis structures.

**Neurological results**

Grading of motor disturbance: On days 1, 3, 5 and 10 the difference regarding the motor function scores between all groups remains significant ( $p < 0.05$ ) (Table 1). When binary comparisons of the groups were evaluated it has been seen that in duration of the whole test, group 2 (trauma) and group 3 (CSA) were not able to reach the scores of the SHAM group regarding the motor scores ( $p < 0.05$ ). But starting from day 5, a significant difference was observed in favour of group 3 when group 2 was compared with group 3 ( $p: 0.002$ ) and this difference got more prominent on day 10 ( $p: 0.001$ ) (Table 2 and Graph 1). According to the in-group analysis of the groups with Wilcoxon test, only group 3 (CSA) had significant difference regarding the day 1 and 10 comparisons (Table 3).

**Table 1.** Comparison of the three groups on 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 10<sup>th</sup> days regarding the Motor Function Scores, by Kruskal-Wallis test ( $p < 0.05$ ).

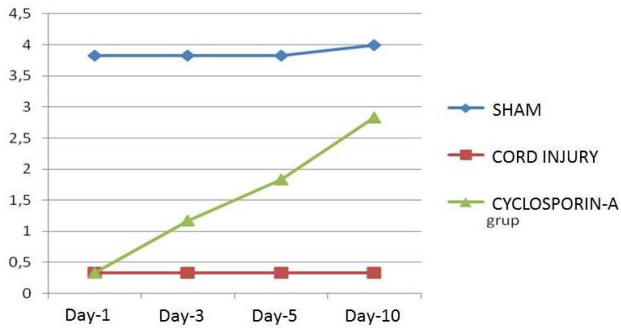
Groups	Sham		Trauma		Cyclosporin-A		Comparison of the groups by Kruskal-Wallis test p
	mean	St error	mean	St error	mean	St error	
MS-1	3.83	0.167	0.33	0.211	0.33	0.211	0.002
MS-3	3.83	0.167	0.33	0.211	1.17	0.307	0.001
MS-5	3.83	0.167	0.33	0.211	1.83	0.167	0.000
MS-10	4	0	0.33	0.211	2.83	0.167	0.000

**Table 2.** In-group comparison of the Motor Score data by Wilcoxon test.

Groups	Motor score 1	Motor score 3	Motor score 5	Motor score 10	Necrosis	Number cells	Apoptotic cells
Groups 1, 2	0.002	0.002	0.002	0.002	0.002	0.003	
Groups 1, 3	0.002	0.003	0.002	0.001	0.002	0.003	
Groups 2, 3	1	0.057	0.004	0.002	0.014	0.008	

**Table 3.** In-group comparison of the Motor Score data by Wilcoxon test.

Group		Motor Score 3- Motor Score 1	Motor Score 5 - Motor Score 1	Motor Score 10 - Motor Score 1	Motor Score 5 - Motor Score 3	Motor Score 10 - Motor Score 3	Motor Score 10 - Motor Score 5
SHAM	Z	.000 <sup>b</sup>	.000 <sup>b</sup>	-1.000 <sup>c</sup>	.000 <sup>b</sup>	-1.000 <sup>c</sup>	-1.000 <sup>c</sup>
	Asymp. Sig. (2-tailed)	1.000	1.000	.317	1.000	.317	.317
Cord injury	Z	.000 <sup>b</sup>	.000 <sup>b</sup>	.000 <sup>b</sup>	.000 <sup>b</sup>	.000 <sup>b</sup>	.000 <sup>b</sup>
	Asymp. Sig. (2-tailed)	1.000	1.000	1.000	1.000	1.000	1.000
Cord injury+Cyclosporin-A	Z	-2.236 <sup>c</sup>	-2.251 <sup>c</sup>	-2.251 <sup>c</sup>	-2.000 <sup>c</sup>	-2.271 <sup>c</sup>	-2.449 <sup>c</sup>
	Asymp. Sig. (2-tailed)	.025	.024	.024	.046	.023	.014

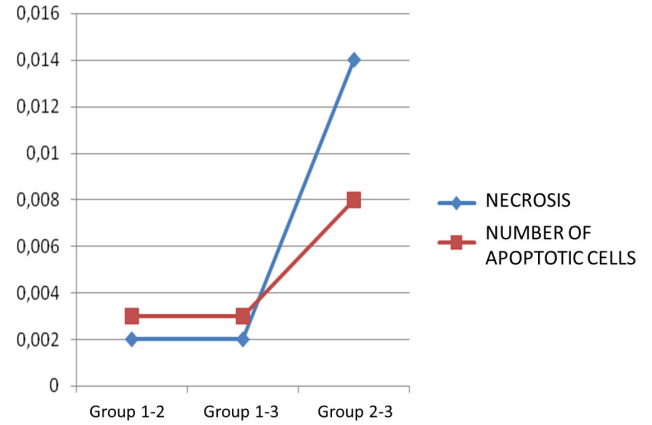


**Graph 1.** Comparison of the five groups on 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> and 10<sup>th</sup> days regarding the Motor Function Scores, by Kruskal-Wallis test.

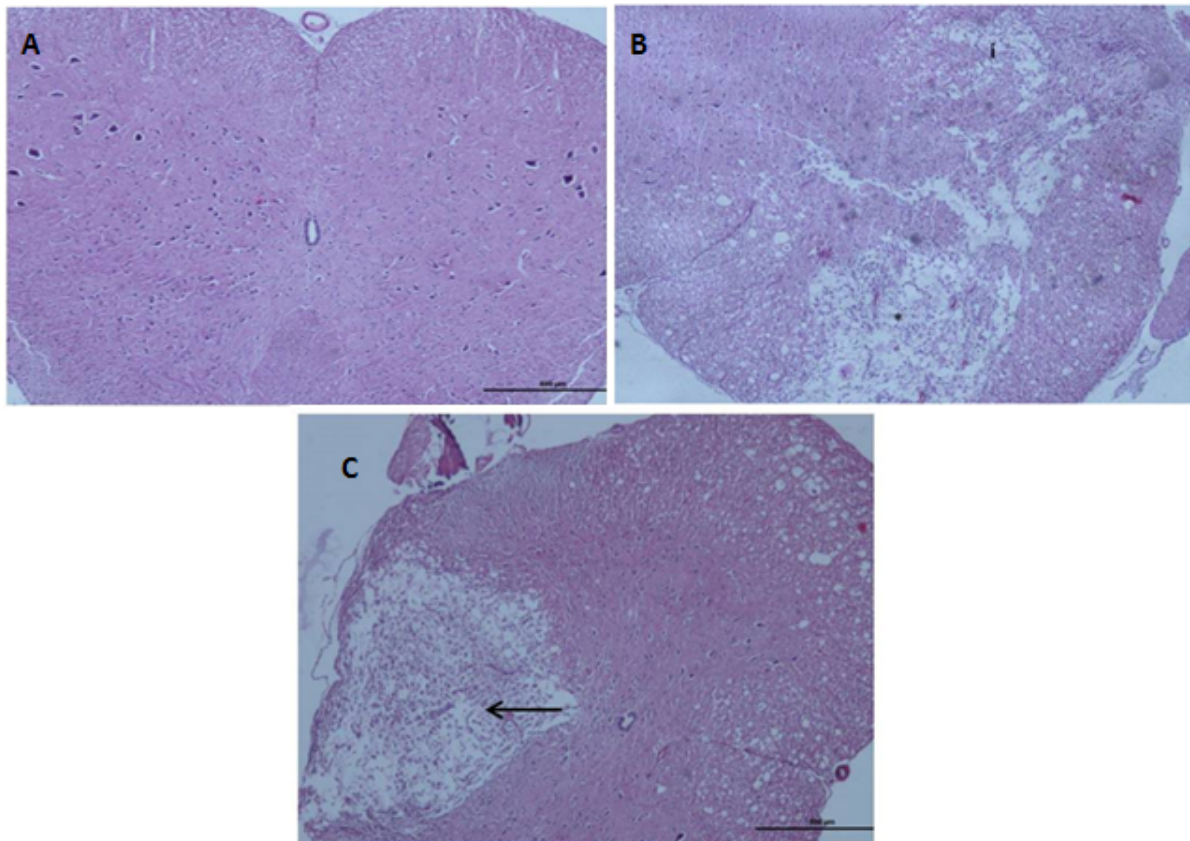
**Histological results**

Light microscopy results: According to the Hemotoxylin-Eosin dye staining necrosis results on day 10, there was a significant difference between all groups (p: 0.00; p<0.01) (Table 2). It was seen that there is a high level of significant difference in binary comparison of the groups according to the necrosis scores, between Group 1 (control-Sham) and other groups (Figure 1). When Group 2 was compared with group 3 (CSA)

it was noted that groups had statistically significance difference, in favor of treatment group (p: 0.008) (Table-2; Graph-2). Briefly cyclosporin-A causes a statistical difference in prevention of necrosis when compared with the trauma group.



**Graph 2.** Comparison of the groups regarding Necrosis and Apoptotic cell numbers.

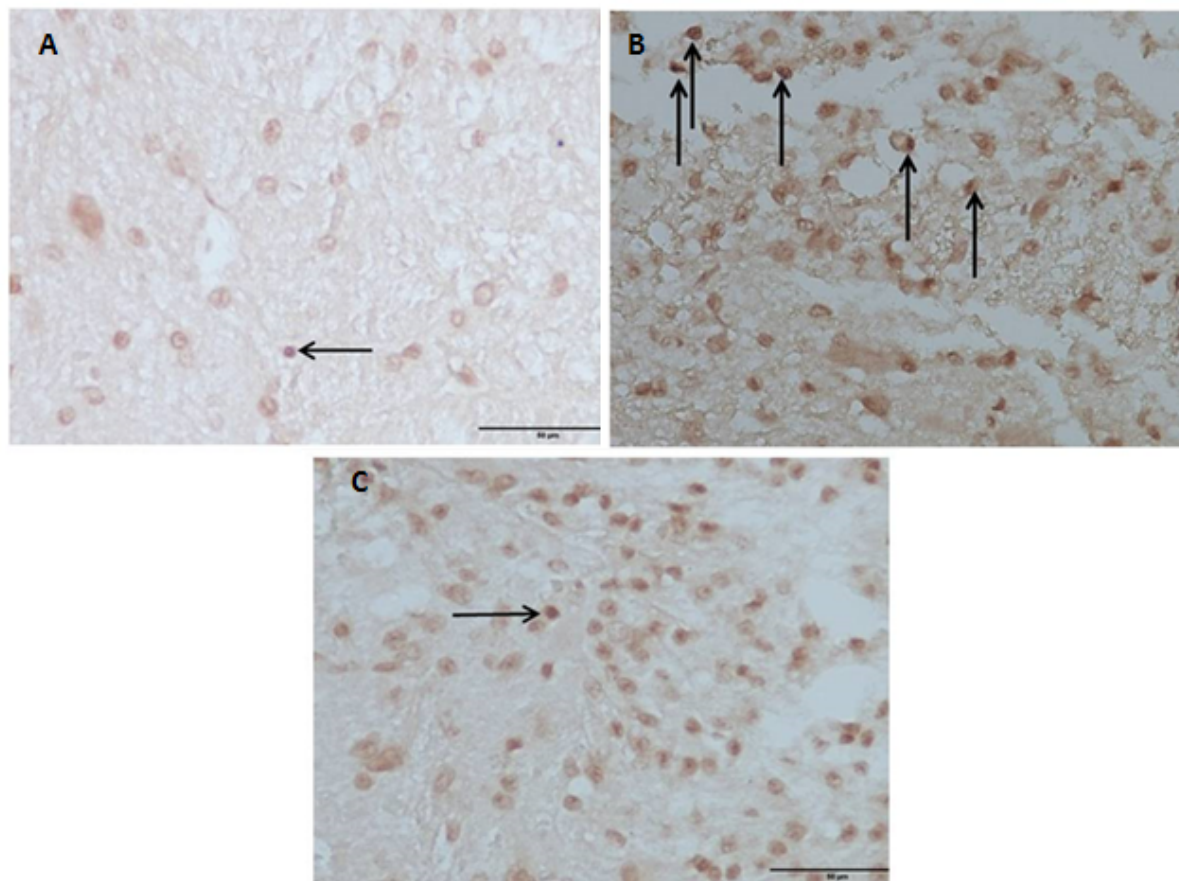


**Figure 1.** a: A section from the Sham group. Hematoxylin-Eosin. b: An image from the spinal cord injury group. Loss of tissue integrity, edema (\*) and increase in number of inflammatory cells. (i) is seen on the lesion site-core area. Hematoxylin-Eosin. C: A section from the Cyclosporin-A group after the spinal cord injury. It can be seen that lesion site-core area is more limited (→). Hematoxylin-Eosin.

### **Terminal deoxynucleotidyltransferase-Mediated UTP end labeling assay (TUNEL) results**

Outcomes of TUNEL on day 10 pointed out that there was a statistical difference in between all groups (Table 2). On day 10 comparing the number apoptosis in binary groups, a significant difference between Group 1 and other groups was seen (Figure

2). Also when group 2 (trauma) and group 3 (CSA) are compared a significant statistical difference in favor of therapy group is seen ( $p: 0.008$ ) (Table 2 and Graph 2). In preventing the apoptosis Cyclosporin-A has a significant difference when compared with the trauma group.



**Figure 2. a:** Apoptotic cells on a section from the Sham group ( $\rightarrow$ ) TUNEL. **b:** Increased number of apoptotic cells on a section from spinal cord injury group can be seen ( $\rightarrow$ ) TUNEL. **c:** On a section from Cyclosporin-A group it can be seen that the number of apoptotic cells are fewer than the injury group ( $\rightarrow$ ) TUNEL.

### **Discussion**

Immunosuppressive agents can be divided into four major groups: 1. T-cell blockers (Cyclosporin-A, Tacrolimus (FK506), Sirolimus), 2. Glucocorticoids, 3. Cytotoxic drugs, 4. Anticore Markers (reagent). As a T-cell blocker, Cyclosporin-A is the most frequently and effectively used agent as an immunosuppressive drug. Cyclosporin-A is a cyclic polypeptide, composed of 11 amino acids and derived from a fungus named as *Tolypocladium inflatum* Gams. Following the recognition of the antigen, cyclosporin-A creates immunosuppression by preventing the early stages of the autoimmune response. It cannot treat an autoimmune response, which occurred previously, or an occurred rejection reaction; but it may prevent a response or reaction if it is given prior to occurrence. It selectively inhibits inducer/helper subtype T lymphocytes (CD4+) and blocks their proliferation and differentiation [14]. It has no effect on the mature T cells. So it should be used as

soon as possible after the encounter with an antigen (for example in an allogeneic transplantation), preferentially in the first 24 hours [14]. Therefore we have administered the treatment right after the trauma. It has its immunosuppressive and secondary neuronal injury preventive effect on CD4+ T lymphocytes by inhibiting calcineurin, which is a  $Ca^{++}$  depended enzyme and one of the steps of the signalling cascade that is initiated by stimulation of the T lymphocyte receptor with antigen. In this chain of reactions, cyclosporin-A inhibits  $Ca^{++}$  transportation via mitochondria membrane transportation (MMT) which is located on the mitochondria membrane. In order to have this effect cyclosporin-A should bind to cyclophilin, which is a cytoplasmic receptor. Binding of cyclosporin-A to cyclophilin causes a protein complex which inhibits activation of calcium/calmodulin dependent calcineurin. This complex inhibits calcineurin or protein phosphatase 2B [14]. If this calcineurin is not inhibited it causes dephosphorylation of nuclear factor of activated T-cells

(NTF) which regulates the release of cytokines like IL-2, IL-3, GM-CSF (Granulocyte colony stimulating factor) and TNF- $\alpha$  [15]. Main clinical effect of cyclosporin-A is probably the inhibition of IL-2 which is produced by T-cells; that which is inhibiting IL-2 that induces proliferation of other T-cells.

An important stage of inflammation after neural trauma is the activation of microglia and leucocyte infiltration [16]. Microglial cells are known to be the immunity cells of the central nervous system (CNS). After infiltration of the ischemic tissue by microglia and astrocytes, cytokines (IL-2, IL-3, TNF- $\alpha$ ), which are major indicators of the inflammatory response, are released [17]. Cyclosporin-A is defined to be effective in inhibition of TNF- $\alpha$  and cyclooxygenase-2 expression from these microglial cells [18]. From the functional perspective, interestingly cyclosporin-A reduces the formation of NO on the microglial cell line and also probably the cytostatic mechanism is effected as well [19]. Beyond this, following the stimulation of the receptor ligands of the peripheral benzodiazepines, production of free radicals on different microglial cell lines are blocked by cyclosporin-A [20].

Also cyclosporin-A's effect extends even through astrocytes since it is known that cyclosporin-A prominently reduces astrocytic apoptosis and the release of TNF- $\alpha$ , IL-1 and IL-2 from astrocytes [21,22].

Cyclosporin's effects on acute CNS disorders like cerebral ischemia was first studied on animal experimental models in the early 1990's. This pioneer studies revealed the useful results of cyclosporin on transient ischemic forebrain injury in rats; cyclosporin was reported to reduce the infarct and edema volume [23]. But, it is very rare for cyclosporin to be used in experimental spinal cord injury and acute brain injury models; but beneficial results were reported. For example, the use of cyclosporin after complete thoracic spinal cord cuts in the rats, axonal regeneration is shown to increase and a decrease in inflammatory effects of macrophages and microglial cells was reported [24]. Same group of investigators later on reported a recovery in functional levels with cyclosporin treatment in rats with complete thoracic spinal cord cuts [25]. In studies on rats with traumatic brain injury, which investigate cyclosporin's positive effects on neurons, axonal degeneration models revealed that mitochondrial swelling, deposition of  $\beta$ -amyloid precursor proteins and compaction of the neurofilaments were reduced [26,27]. Cyclosporin-A application in first 6 hours, in thoracic cord injury models, was shown to inhibit lipid peroxidation and enhancement in the mobility of the rats exposed to lesion [28]. On the other hand in a more recent study objected these findings, reporting no reduction in tissue loss in groups which were treated with cyclosporin and no enhancement in the motor scores [29]. But later on it has been claimed that this failure of the drug was due to insufficient dosing of the cyclosporin [30]. Most lately a study in 2010, reported that cyclosporin, which is used after transportation of oligodendrocyte precursor cells (OPC) to the traumatized

spinal cord, had better histological outcomes and in correlation to this better functional recovery results were achieved by cyclosporin, when compared to the control group; but cyclosporin failed to prevent the transported oligodendrocyte precursor cells to transform into astrocytes [31]. In fluid-percussion injury models cyclosporin-A was reported to improve motor and amnesic functions in traumatic brain injury [32]. Reports, that explaining cyclosporin's effects on isolated neurons are very rare. But studies on experimentally lesioned neuroblastoma cells showed that cyclosporin prevents cells from apoptosis, inhibits caspase activation and activates neuronal growth [33,34].

Phase-II clinical researches on traumatic brain injuries are being carried out and those trials are showing the safety of cyclosporin-A. But there is a heterogeneity regarding these trials especially in timing of cyclosporin-A treatment after traumatic brain injury [35].

There is not any clinical study in the literature about cyclosporin-A treatment in traumatic spinal cord injuries yet. And to our knowledge, no reports of clinical use of cyclosporin-A in acute CNS disorders (such as ischemia) exist. By *in vitro* and *in vivo* evaluations of these findings it is accepted that Cyclosporin-A has direct inhibitor effects on microglial cells, it has neuroprotective and neuroregenerative characteristics and cyclosporin-A has direct effects on neurons and glial cells [36].

As mentioned above there are no experimental studies in the literature, which match identically to our study. And also the number of experimental spinal cord injury studies with cyclosporin, are very limited. Palladini et al. [25] showed that groups which were treated with cyclosporin-A, had structural and functional recovery, in thoracic cord injuries. Also Diaz-Ruiz et al. [28] declared that in groups treated with cyclosporin-A after thoracic cord contusion, lipid peroxidation was decreased. But in contrast to these outcomes Rabchevsky et al. [29] reported that in both groups, treated with cyclosporin-A or not, had similar results in functional and structural recovery parameters.

According to the out-comes of the motor score recovery in both trauma groups were not significant up to day 5; whereas after day 5 in favor of "cord injury+cyclosporin group" there was a significant recovery result (Table 2). Also, when groups were analyzed with binary comparison, (Mann-Whitney U test) functional evaluation (motor score) and also structural evaluation (histologically necrosis-apoptosis) had statistically significant outcomes in favor of "cord injury+CSA" (Table 2).

## Conclusion

In our experimental rat spinal cord injury model; we introduce that cyclosporin-A has neuroprotective (Necrosis/Apoptosis data in favor of cyclosporin-A group) and regenerative effects (motor score in favor of cyclosporin-A group) on secondary spinal cord injury, with statistically significant data.

## References

1. Hausmann ON. Post-traumatic inflammation following spinal cord injury. *Spinal Cord* 2003; 41: 369-378.
2. Beck KD, Nguyen HX, Galvan MD, Salazar DL, Woodruff TM, Anderson AJ. Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. *Brain* 2010.
3. Fleming JC, Norenberg MD, Ramsay DA, Dekaban GA, Marcillo AE, Saenz AD, Pasquale-Styles M, Dietrich WD, Weaver LC. The cellular inflammatory response in human spinal cords after injury. *Brain* 2006; 129: 3249-3269.
4. Hamada Y, Ikata T, Katoh S, Nakauchi K, Niwa M. Involvement of an intercellular adhesion molecule 1-dependent pathway in the pathogenesis of secondary changes after spinal cord injury in rats. *J Neurochem* 1996; 66: 1525-1531.
5. McTigue DM, Tani M, Krivacic K, Chernosky A, Kelner GS. Selective chemokine mRNA accumulation in the rat spinal cord after contusion injury. *J Neurosci Res* 1998; 53: 368-376.
6. Pineau I, Lacroix S. Proinflammatory cytokine synthesis in the injured mouse spinal cord: multiphasic expression pattern and identification of the cell types involved. *J Comp Neurol* 2007; 500: 267-285.
7. Bethea JR, Dietrich WD. Targeting the host inflammatory response in traumatic spinal cord injury. *Curr Opin Neurol* 2002; 15: 355-360.
8. Bethea JR, Castro M, Keane RW, Lee TT, Dietrich WD. Traumatic spinal cord injury induces nuclear factor-kappaB activation. *J Neurosci* 1998; 18: 3251-3260.
9. Conti A, Cardali S, Genovese T, Di Paola R, La Rosa G. Role of inflammation in the secondary injury following experimental spinal cord trauma. *J Neurosurg Sci* 2003; 47: 89.
10. Fitch MT, Silver J. CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. *Exp Neurol* 2008; 209: 294-301.
11. Taoka Y, Okajima K, Uchiba M, Murakami K, Kushimoto S. Role of neutrophils in spinal cord injury in the rat. *Neuroscience* 1997; 79: 1177-1182.
12. Drummond JC, Moore SS. The influence of dextrose administration on neurologic outcome after temporary spinal cord ischemia in the rabbit. *Anesthesiology* 1989; 70: 64-70.
13. Black P, Markowitz RS, Cooper V, Mechanic A, Kushner H. Models of spinal cord injury: Part 1. Static load technique. *Neurosurgery* 1986; 19: 752-762.
14. Kayaalp S. Rasyonel tedavi yonunden tibbi farmakoloji. Ankara, Hacettepe-Tas. 2002.
15. Rao A, Luo C, Hogan PG. Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 1997; 15: 707-747.
16. Gong C, Hoff JT, Keep RF. Acute inflammatory reaction following experimental intracerebral hemorrhage in rat. *Brain Res* 2000; 871: 57-65.
17. Iadecola C, Forster C, Nogawa S, Clark HB, Ross ME. Cyclooxygenase-2 immunoreactivity in the human brain following cerebral ischemia. *Acta Neuropathol* 1999; 98: 9-14.
18. Choi HB, Khoo C, Ryu JK, Van Breemen E, Kim SU, McLarnon JG. Inhibition of lipopolysaccharide-induced cyclooxygenase-2, tumor necrosis factor- $\alpha$  and  $[Ca^{2+}]_i$  responses in human microglia by the peripheral benzodiazepine receptor ligand PK11195. *J Neurochem* 2002; 83: 546-555.
19. Lockhart BP, Cressey KC, Lepagnol JM. Suppression of nitric oxide formation by tyrosine kinase inhibitors in murine N9 microglia. *Br J Pharmacol* 1998; 123: 879-889.
20. Jayakumar A, Panickar K, Norenberg M. Effects on free radical generation by ligands of the peripheral benzodiazepine receptor in cultured neural cells. *Journal of neurochemistry*. 2002; 83: 1226-1234.
21. Pyrzynska B, Lis A, Mosieniak G, Kaminska B. Cyclosporin A-sensitive signaling pathway involving calcineurin regulates survival of reactive astrocytes. *Neurochem Int* 2001; 38: 409-415.
22. Herman ZS. Immunophilin ligands decrease release of pro-inflammatory cytokines (il-1b, tnf- $\alpha$  and il-2) in rat astrocyte cultures exposed to simulated ischemia in vitro. *Pol J Pharmacol* 2004; 56: 129-136.
23. Shiga Y, Onodera H, Matsuo Y, Kogure K. Cyclosporin A protects against ischemia-reperfusion injury in the brain. *Brain Res* 1992; 595: 145-148.
24. Teichner A, Morselli E, Buttarelli F, Caronti B, Pontieri F, Venturini G. Treatment with cyclosporine A promotes axonal regeneration in rats submitted to transverse section of the spinal cord. *Journal fur Hirnforschung* 1992; 34: 343-349.
25. Palladini G, Caronti B, Pozzessere G, Teichner A, Buttarelli F, Morselli E. Treatment with cyclosporine A promotes axonal regeneration in rats submitted to transverse section of the spinal cord--II--Recovery of function. *Journal fur Hirnforschung*. 1995; 37: 145-153.
26. Büki A, Okonkwo DO, Povlishock JT. Postinjury cyclosporin A administration limits axonal damage and disconnection in traumatic brain injury. *J Neurotrauma* 1999; 16: 511-521.
27. Okonkwo DO, Povlishock JT. An intrathecal bolus of cyclosporin A before injury preserves mitochondrial integrity and attenuates axonal disruption in traumatic brain injury. *J Cereb Blood Flow Metab* 1999; 19: 443-451.
28. Diaz-Ruiz A, Rios C, Duarte I, Correa D, Guizar-Sahagun G. Cyclosporin-A inhibits lipid peroxidation after spinal cord injury in rats. *Neurosci Lett* 1999; 266: 61-64.
29. Rabchevsky AG, Fugaccia I, Sullivan PG, Scheff SW. Cyclosporin A treatment following spinal cord injury to the rat: behavioral effects and stereological assessment of tissue sparing. *J Neurotrauma* 2001; 18: 513-522.

30. Ibarra A, Hauben E, Butovsky O, Schwartz M. The therapeutic window after spinal cord injury can accommodate T cell-based vaccination and methylprednisolone in rats. *Eur J Neurosci* 2004; 19: 2984-2990.
31. Lü HZ, Wang YX, Zhou JS, Wang FC, Hu JG. Cyclosporin A increases recovery after spinal cord injury but does not improve myelination by oligodendrocyte progenitor cell transplantation. *BMC neurosci* 2010; 11: 127.
32. Alessandri B, Rice AC, Levasseur J, DeFord M, Hamm RJ, Bullock MR. Cyclosporin A improves brain tissue oxygen consumption and learning/memory performance after lateral fluid percussion injury in rats. *Journal of neurotrauma*. 2002; 19: 829-841.
33. Capano M, Virji S, Crompton M. Cyclophilin-A is involved in excitotoxin-induced caspase activation in rat neuronal B50 cells. *Biochem J* 2002; 363: 29-36.
34. Sheehan J, Eischeid A, Saunders R, Pouratian N. Potentiation of neurite outgrowth and reduction of apoptosis by immunosuppressive agents: implications for neuronal injury and transplantation. *Neurosurg foc* 2006; 20: 1-7.
35. Lulic D, Burns J, Bae EC, van Loveren H, Borlongan CV. A review of laboratory and clinical data supporting the safety and efficacy of cyclosporin A in traumatic brain injury. *Neurosurgery* 2011; 68: 1172-1185.
36. Hailer NP. Immunosuppression after traumatic or ischemic CNS damage: it is neuroprotective and illuminates the role of microglial cells. *Prog Neurobiol* 2008; 84: 211-233.

**\*Correspondence to:**

Seckin Emre Cancan

Department of Neurosurgery

Abant Izzet Baysal University School of Medicine, Bolu,

Turkey