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Original Investigation

The Effect of Quetiapine on Treatment of Experimental Acute Spinal Cord Injury

Murat Hamit AYTAR¹, Soner CIVI², Memduh KAYMAZ³, Ertan ERGUN⁴, Fevziye Figen KAYMAZ⁵, Aydın PASAOGLU³

¹Acibadem University, Vocational School of Health Services, Department of Neurosurgery, Istanbul, Turkey

²Başkent University, Faculty of Medicine, Department of Neurosurgery, Adana, Turkey

³Gazi University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey

⁴Kavaklıdere Umud Hospital, Neurosurgery Clinic, Ankara, Turkey

⁵Hacettepe University, Faculty of Medicine, Department of Neurosurgery, Ankara, Turkey

ABSTRACT

AIM: It is well known that treatment modalities against secondary damage due to spinal cord injury (SCI) are very important. This phase has been researched in many experimental studies. Apoptosis is one of the major mechanisms of secondary damage on spinal cord. The present study was undertaken to determine if quetiapine, a 5-HT₂ receptor blocker atypical antipsychotic agent can rescue neuronal cells from apoptosis in a SCI model.

MATERIAL and METHODS: Thirty-two female Wistar rats were separated to 4 equal groups. Total laminectomy was performed at T5-7 level and spinal cord injury was produced by using the clip compression technique. Each rat from groups "1 day" (D-I) and "7 days" (D-II) was daily injected intraperitoneally with Quetiapine (10 mg/kg/day). No treatment was administered to the control groups "1 day" (K-I) and "7 days" (K-II). At the end of follow-up periods, all animals were sacrificed and spinal cords were removed. Apoptotic cells were evaluated by using immunohistochemical technique (TUNEL) in injured spinal cord specimens.

RESULTS: There was a statistically significant difference while counting ApopTag positive cells, both at 1 day groups of K-I and D-I ($p=0.0000008$) and at 7 day groups of K-II and D-II ($p=0.000005$). Unlike the 1-day period, a statistically significant difference was found between grey and white matter ApopTag positive cells at the 7th day ($p=0.0001$).

CONCLUSION: Quetiapine has a protective effect on secondary damage caused by SCI, while also can be used in post-traumatic stress disorder, depression and agitation as a versatile agent.

KEYWORDS: Spinal cord injury, Quetiapine, Apoptosis, Secondary damage

INTRODUCTION

Spinal cord injury (SCI) is a catastrophic and common neurological disorder that has intense influences on modern life concerning physical, psychosocial, and socioeconomic perspectives. SCI is generally accepted to be a two-step process involving primary and secondary injury mechanisms. Acute spinal cord injury is characterized by primary axonal mechanical injury and secondary

damage induced by different biochemical reactions involving also tissues surrounding the primary injured area. Pathophysiological mechanisms are essential to understand and manage spinal cord injury. Spinal cord may become damaged by the "primary mechanical injury" and damage will expand with time because of the activation of pathophysiological mechanisms called "secondary cord injury". Secondary injury of SCI may result from spinal cord edema, ischemia, free radical damage, electrolyte imbalance,

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Corresponding author: Soner CIVI

E-mail: sonercivi@yahoo.com

excitotoxicity, inflammatory injury, and apoptosis (9). Various studies have investigated pharmacologic agents, such as methylprednisolone, melatonin, erythropoietin, magnesium, mexiletine, naloxone, infliximab, clotrimazole, lamotrigine and hyperbaric oxygen, that protect against or reduce the secondary injury after experimental SCI and among these only methylprednisolone has been shown to provide benefit in large clinical trials (4,11,13-16,23,24,26).

The active ingredient quetiapine that we used in our study has a serotonin antagonist effect. Serotonin has multiple through 7 different families of receptors (5-HT1 to 5-HT7). Quetiapine is a 5-hydroxytryptamine-2 (5-HT2) receptor antagonist. 5-HT2 receptors occupies in the cells of the blood vessel wall, myocardium, thrombocytes, mast cells and central nervous system cells widely. It is well known that serotonin also plays role in injured tissues, inflammation and inflammatory pain. Also studies of 5-HT2 receptor blockers have shown that they might be effective in the treatment of lumbar pain secondary to discopathy.

It has even been claimed that 5-HT2 blockers can provide equal pain control as non-steroid anti-inflammatory drugs (NSAIDs) (10,12).

Serotonin may also act as mediator through 5-HT2 receptors in cardiac injury due to ischemic reperfusion. In some experimental studies on the subject, it has been concluded that 5-HT2-receptor blockage prevents cellular injury due to myocardial ischemia (19). Rajesh et al. have also presented that, 5-HT2 receptor blocker sarpogrelate was preventing down regulation of anti-apoptotic protein Bcl-2 and protecting the heart against ischemia-reperfusion injury (21).

Experimental clinical studies have also shown that a dibenzothiazepine derivative, serotonin receptor blocker atypical antipsychotic drug quetiapine was also suitable for treatment of aggression and agitation due to traumatic brain injury (17).

In the light of these data, we believed that quetiapine while also averting the neuropsychiatric complications of the trauma, could show a neuroprotective effect and decrease apoptotic cell death and negative effects secondary to injury of the spinal cord via 5-HT2 receptors.

■ MATERIAL and METHODS

Thirty-two male, adult Wistar rats weighing between 250 and 300 g were used in this study. Four equal groups each containing 8 randomly selected rats were constituted. All the rats were obtained from the Gazi University, Experimental Animals Research Center (GUDAM) and housed in the same center. All animals were kept under controlled light in dark conditions and housed 7 days before the tests for adaptation. There was no water and food deprivation. All experiments were approved by our Institutional Review Board and performed in accordance with the local guidelines to minimize animal discomfort.

Groups

The same surgical procedures were performed on each four groups;

Group 1 (K-I): 1 day survived control group (n=8)

Group 2 (D-I): 1 day survived treatment group (n=8)

Group 3 (K-II): 7 days survived control group (n=8)

Group 4 (D-II): 7 days survived treatment group (n=8)

Anesthesia and Surgical Procedure

Anesthesia was induced by intramuscular administration of 50 mg/kg ketamine hydrochloride (Ketalar, Pfizer; Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer; Istanbul, Turkey). The rats were numbered with ear tags. Their mid-backs were shaved and cleaned with 10% polyvinylpyrrolidone/iodine. The medication groups were administered quetiapine 10 mg/kg/day intraperitoneally 30 minutes after the trauma was caused, and then once a day at the same time for 7 days. All surgical procedures were performed under the microscope (Opmi 99, Carl Zeiss, Germany). Th5-Th7 total laminectomy was performed by the same surgeon with a standard surgical procedure to each animal (8,20,22). Acute spinal injury was obtained under sterile condition with clip compression technique at level Th6 thoracic spinal cord. The spinal cord was compressed for 60 seconds by Yaşargil aneurysm clip (Figure 1). After the surgical procedure, all the animals were paraplegic.

After the surgical procedures, quetiapine was administered 10 mg/kg intraperitoneally to animals of the D-I and D-II groups at 30th minute of the operation. The control groups (K-I and K-II) received no treatment. First day groups (D-I and K-I) were sacrificed at 24th hour of the trauma. Laminectomy sites were enlarged and Th6 cord trauma level resected with expanding 5 mm proximally and distally. The animals of groups of K-II and D-II were followed up for 7 days. Quetiapine was administered daily to each rat (10 mg/kg IP). After 7 days, all animals of group K-II and D-II were sacrificed and their injured cords were resected with the same procedure.

Immunohistochemical Procedure

All the spinal cord samples were fixed with formalin and embedded in paraffin blocks. Sections with 5 µm thickness were obtained from paraffin blocks on special sialinized slides. Following deparaffinization, some samples were stained with haematoxylin-eosin and the accuracy of the field was checked. Other sections were marked with the immunohistochemical TUNEL method by using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, USA cat#S7101). Background staining was achieved by methyl green. After dehydration with 100% n-butanol, all slides were closed using closure balsam.

Sample Evaluation

All the immunohistochemically stained samples were analyzed by light microscopy (Leica Microsystems RM6000, Germany). The cells with brown nuclear staining, over the methylgreen-stained light green background were considered ApopTag positive. On each slide, four randomly selected areas (two from gray matter, two from white matter) were evaluated under x40 magnification. ApopTag positive cells were counted by using ocular grid and noted for each group separately. The apoptotic cells of the groups were shown in Figure 2A, B and Figure 3A, B.

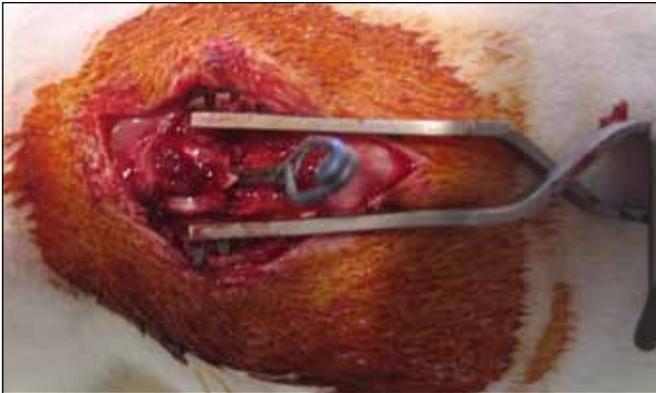


Figure 1: The photo of the clip compression technique by Yasargil aneurysm clip at level Th6 thoracic spinal cord.

Additionally, all the samples stained with haematoxylin–eosin in each group were evaluated under the light microscope with x50 and x100 magnification to examine the tissue integrity and characteristics (Figure 4).

Statistical Method

Differences between groups regarding cells were analyzed using two-way analysis of variance. P value <0.05 was considered as statistically significant.

RESULTS

ApopTag positive cells of grey and white matter were counted one by one and evaluated with immunohistochemical staining. Statistical analysis results are shown in Table I. On day 1

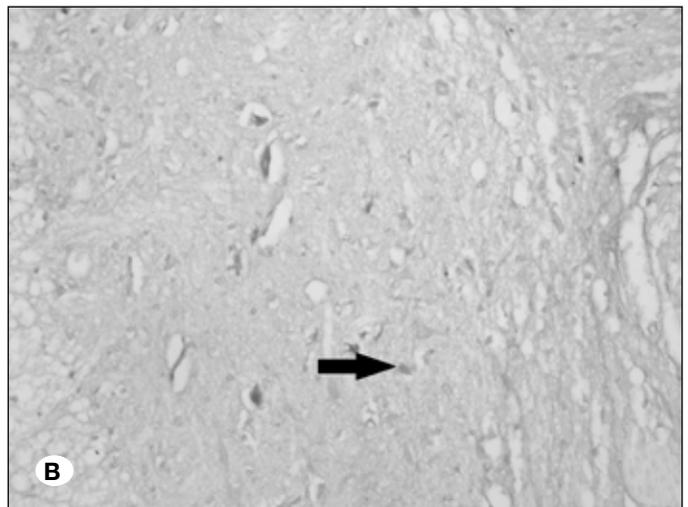
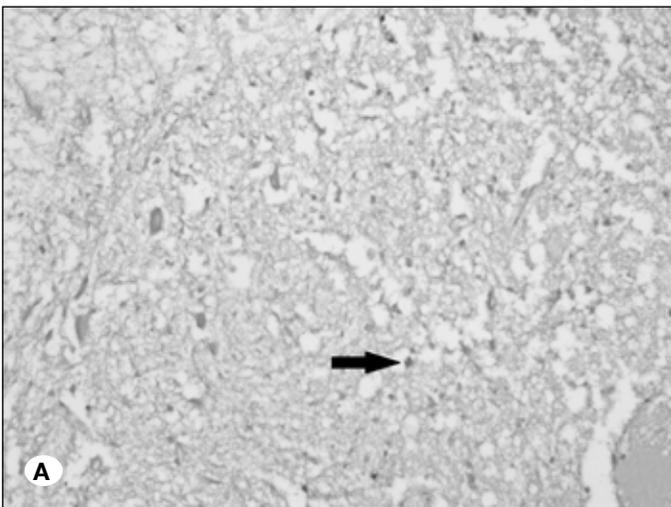


Figure 2: A) In the control group (K-I), apoptag positive cells can be seen in brown color during the first day as a result of tissue integrity impairment. Black arrow indicates apoptotic cell sample (original magnification 20x). **B)** Less apoptag positive cells can be seen in the first day of the experimental group (D-I) compared to the control group. Black arrow indicates apoptotic cell sample (original magnification 20x).

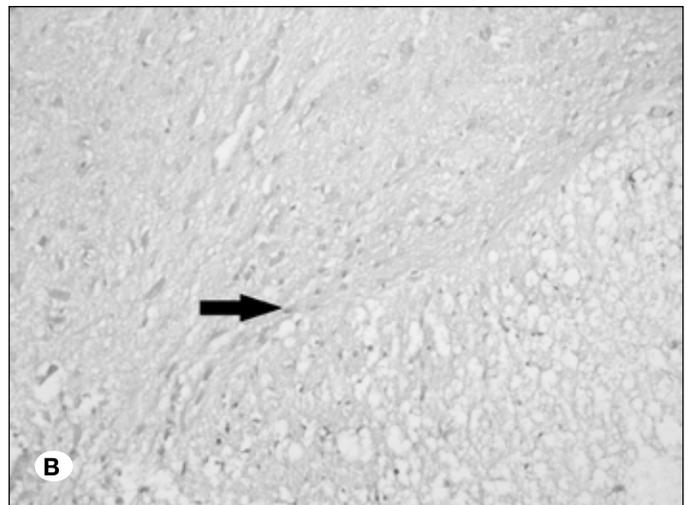
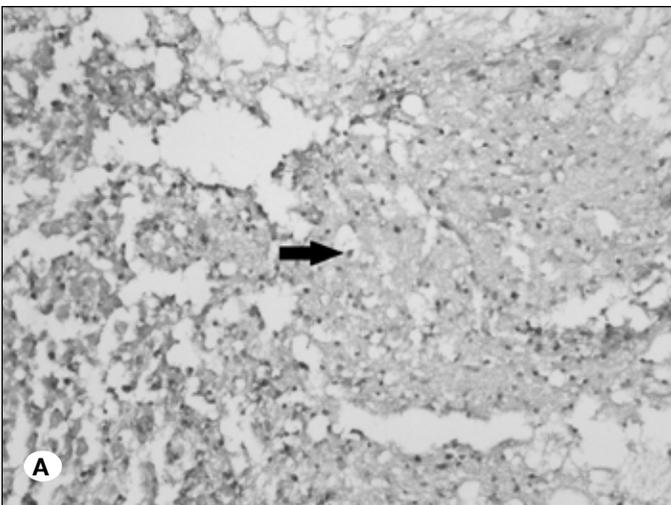


Figure 3: A) Many apoptag positive, brown colored cells can be seen on the tissue impaired cord on the 7th day of the control group (K-II). Black arrow indicates apoptotic cell sample (original magnification 20x). **B):** The lower number of positive apoptag cells in the 7th day research group (D-II) compared to the control group was noticeable. Black arrow indicates apoptotic cell sample (original magnification 20x).

there was a statistically significant ($p=0.0000008$) difference between the control group and the study group concerning ApopTag positive cells but no significant difference was found between grey matter and white matter ApopTag positive cells ($p=0.216$). On day 7 there was also a statistically significant difference concerning ApopTag positive cells of the control group and the study group ($p=0.000005$), and unlike the acute period statistically significant difference was found between grey and white matter ApopTag positive cells ($p=0.0001$). Figure 5 shows the comparison of average apoptotic cell numbers at the first and seventh days of the control group and the research group in the grey matter, white matter and all areas.

Apoptotic cell counts were also evaluated one by one for the grey matter, and for the white matter separately. Cellular and group (control-study group) interaction in the acute period between control (K-I) and study (D-I) groups were statistically

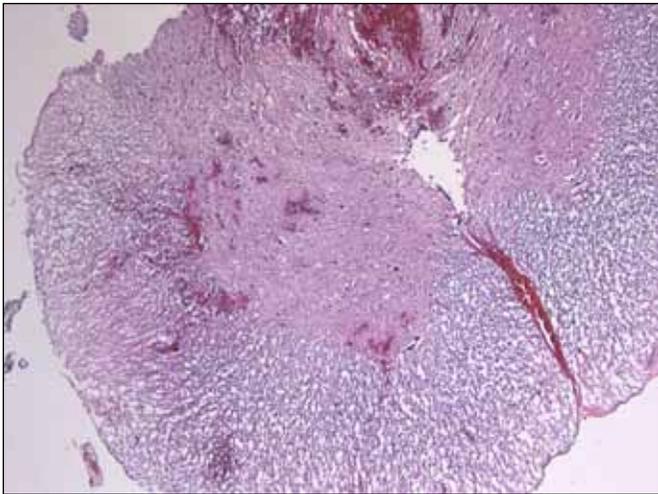


Figure 4: After the experimental injury, spinal cord section stained with haematoxylin–eosin shows traumatic injury and bleeding areas under the light microscope with x50 magnification.

insignificant ($p=0.889$) but (K-II and D-II) cellular and group (control-study group) interaction in the subacute period were found to be statistically significant ($p=0.002$).

It was observed that quetiapine provided better protection on grey matter cells than white matter cells in the subacute period. This condition was also observed on immunohistochemical staining as presence of more apoptotic cells in the white matter compared to the grey matter.

DISCUSSION

Spinal cord injury can be seen at any age, and it is an important medical and social problem. The victims are usually at the second or third decades of their lives (9). Many studies have focused on the secondary injury mechanism of SCI. It has been suggested that after SCI, apoptosis is a crucial cause of cell loss that contributes to neurological deficit (2,7,18).

Many studies have reported an important morphological and biochemical evidence of the presence of apoptosis after SCI. Apoptosis occurs in many neurons, oligodendrocytes, microglia and astrocytes. Many weeks after the injury the death of oligodendrocytes in white matter tracts continues, and this may contribute to post-injury demyelination (3). After the role of apoptosis and leading pathways were discovered in secondary injury, many useful agents blocking these pathways were experimentally used. Yune et al. used 17-beta estradiol and experimentally showed its efficacy. In another study, the preventive effect of erythropoietin from apoptosis caused by post-traumatic spinal cord injury has been shown (1,25). Luo and Shi, in a recent experimental study, showed that polyethylene glycol inhibited apoptotic cell death after spinal trauma. It has been previously known that polyethylene glycol shows neuroprotective effects by stabilizing the cellular and mitochondrial membranes and in this study it was found that it inhibits cytochrome c, thus blocking apoptosis (18). A different study showed that sarpogrelate a 5-HT2 receptor blocker, blocked cardiomyocytes from dying through apoptotic ways and prevents postischemic myocardial dysfunction (19,21).

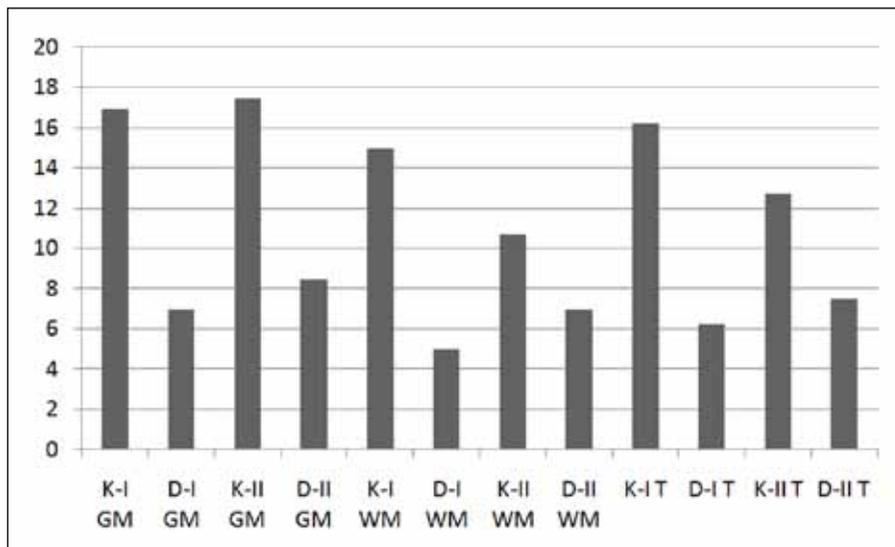


Figure 5: Graphic representation of the average apoptotic cell number of each group in white matter areas, grey matter areas and all areas (**GM:** Grey Matter, **WM:** White Matter, **T:** Total).

Table I: The Number of Apoptotic Cells in 4 Randomly Selected Fields from the Grey and White Matters of Each Animal in the Group

		n	Mean	Std. Deviation	Median	Minimum	Maximum
K-I	Grey matter	8	17.18	2.13	17.00	13.5	20.0
	White matter	8	15.62	3.55	15.00	12.0	23.5
	Total	16	16.40	2.94	16.25	12.0	23.5
D-I	Grey matter	8	7.00	2.93	7.00	3.5	12.0
	White matter	8	5.75	3.70	5.00	1.5	11.5
	Total	16	6.37	3.29	6.25	1.5	12.0
K-II	Grey matter	8	17.68	4.30	17.5	12	26.5
	White matter	8	10.43	1.98	10.75	6.5	13.5
	Total	16	14.06	4.94	12.75	6.5	26.5
D-II	Grey matter	8	7.68	2.03	8.50	3.5	9.5
	White matter	8	6.81	1.06	7.00	5.0	8.0
	Total	16	7.25	1.63	7.5	3.5	9.5

The 5-HT₂-receptor group that is our subject was shown to be related to apoptotic cellular death mechanisms secondary to cranial or spinal ischemia or trauma. Capela et al. have used 3,4-Methylenedioxymethamphetamine (Extacy-MDMA) in their studies in 2006 and 2007 (5,6). It was shown that MDMA had serotonergic effects, especially causing apoptosis through 5-HT₂ receptor pathway. Apoptosis can be prevented by using ketanserin, which is a 5-HT₂ blocking agent (6,21).

Quetiapine is well-known in psychiatry. Besides its wide use in agitation and control of aggression, according to this study it finds a wide use in the post-traumatic brain injury period. It is also used in the treatment of post-traumatic stress disorders. Neurosurgical patients exposed to post-traumatic spinal injury will therefore be able to benefit from the versatile therapeutic use as an auxiliary agent.

Apoptotic pathways constitute a very important mechanism of secondary injury. Our study has shown that quetiapine had a protective effect on apoptotic deaths in cells of secondary injury in our experimental spinal cord injury model created by the clip compression technique. Its effect was related to the blockage of 5-HT₂ receptors, so finally quetiapine seems to have an important protective mechanism. Therefore, quetiapine can be considered in post-traumatic stress disorders, agitation, aggressiveness, and the treatment of depression in patients with SCI and could be a versatile option to treat the damaged cord.

■ CONCLUSION

Quetiapine has a protective effect on secondary damage caused by spinal cord injury, and can also be used in post-traumatic stress disorder, depression and agitation as a versatile agent. However, its routine use in clinical practice

has to be supported by further studies. We believe that studies performed at molecular levels elucidating the effect on neurons and glial cells would be appropriate.

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