COMPARISON OF TRİMETAZİDİNE AND RİLUZOLE TO METİLPREDNİSOLONE İN SPİNAL CORD TRAUMA

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ABSTRACT:

Background*: over the last several decades significant advances have occured in the assesment and management of spinal cord trauma.*

Objective*: evaluate the affects of Riluzole, Trimetazidine and Riluzole-Trimetazidine combination in spinal cord trauma and compare them with the affects of Metil prednisolone in spinal cord trauma.*

Methods*: 49 Wistar Albino rats were used for the study. They were seperated in to seven groups ; control, sham, trauma, metilprednisolone used, riluzole used, trimetazidine used and trimetazidine-riluzole combination used group.*

Results*: In motor function evaluation the group where trimetazidine and riluzole were used together was close to methylprednisolone used group. In histopathological evaluation, necrosis development was significantly reduced in the high-dose methylprednisolone group, but no significant difference was observed in the groups given separately and in combination with riluzole and trimetazidine. In biochemical search total antioxidant status was found to be close to those of trimetazidine and riluzole combined with high doses of methylprednisolone,Total Oxidant Status assessment, the values of the group given high dose methylprednisolone and the group given trimetazidine and riluzole and the groups in which riluzole was given alone were found to be close to each other.*

Conclusions*: In addition to the high-dose methylprednisolone treatment , which has an important place in the current treatment scheme in the treatment of spinal cord trauma , combined use of trimetazidine and riluzole is thought to be beneficial both histopathologically and biochemically.*

Keywords: *spinal cord trauma, Trimetazidine, Riluzole, Metil prednisolone.*

Introduction

Spinal cord trauma is an important disease that affects state policies in terms of social and economic aspects as well as individual and social importance due to its consequences. As a result of spinal cord trauma, individuals who can be able to maintain their own lives in the society, become need of care and cannot survive alone. Therefore, a lot of research and studies have been done for spinal cord trauma and continue to be done. However, an accepted treatment protocol that fully cures cord damage has not yet been developed. (1,2).

In experimental and clinical studies, acute spinal cord trauma has been examined in two separate parts as primary damage caused by mechanical effects of trauma and secondary damage caused by physiopathological changes after primary damage(3). In 1911, Allen et al. Identified secondary injury, causing a change in the

treatment approach so far. Subsequent studies have also suggested that secondary damage that begins with the activation of cell death steps following primary damage from neurological deficits after spinal cord trauma is responsible. (4).

Although there are many studies to prevent spinal cord injury secondary to trauma due to trauma, no treatment has been included in the routine treatment plan except methylprednisolone treatment. (4, 5, 6). Although methylprednisolone is thought to affect lipid peroxidation, free radical formation and edema caused by secondary injury in acute spinal cord injury due to spinal cord trauma, there is still controversy about its mechanism. Young et al. recommended that all treatment approaches for spinal cord injury be compared with the methylprednisolone agonist study (7). There are many studies in the literature comparing the new treatment methods with methylprednisolone treatment and tried the combined treatments. (8, 9, 10, 11).

In this study we evaluated , experimentally traumatic spinal cord injury with 49 rats in animal experiment laboratory with the help of Mustafa Kemal University Scientific Research Projects coordination unit, methylprednisolone, trimetazidine, riluzole and trimetazidine and riluzole combination by applying a combination of control and sham groups with clinical, biochemical and pathological comparison of their activities.

Materials and Methods

Female Wistar Albino rats weighing 250-300gr were used for the study. The rats were kept under constant laboratory conditions of 20-22 *C room temperature, a 12 hour light/dark cycle , and were given free Access to food and water. Prior to surgery the rats receivedan anesthetic pf 10mg/kg xylasine (Bayer Birleşik alman İlaç Fabrikaları T.A.Ş. Istanbul, Turkey) and 60 mg/kg ketamine hydrochloride (Parke Davis Istanbul, Turkey), administirated intramuscularly. The rats were pinned in the prone position. Following T5-T12 midline skin incision spinous process and laminar arcs of T7 and T8 were removed after paravertebral muscle dissection. The Dura was left intact. The trauma was produced with the method which was described by Allen in 1911(2). The force was applied via a titanium rod (9-mm diameter tip, weighing 5gr) that was rounded at the surface, which was contacted to the spinal cord after being dropped vertically through a metal 10 mm diameter and 5 cm height tube.

The rats were randomly allocated in to seven groups; a control group of seven rats in which we neither surgery nor medical treatment performed; Sham operated group in which only laminectomy was performed and nontraumatized spinal cord samples obtained by biochemically and pathologically; Trauma group of seven rats following surgical and traumatic interventions 1 cm injured spinal cord samples were removed at 48 hours posttrauma ; Metilprednisolone group of seven rats in which a 30mg/kg single dose immediately after trauma and 5,5 mg/kg maintenance dose of metilprednisolone was administirated intraperitoneally (Mustafa Nevzat Ilac Sanayii A.S.) and 1 cm injured spinal cord samples and blood samples were removed at 48 hours posttrauma ; Trimetazidine group of seven rats in which 3mg/kg of trimetazidine (Sitorel 20 mg Mustafa Nevzat Ilac Sanayii A.S.) was administirated intraperitoneally twice a day after trauma and 1 cm injured spinal cord samples and blood samples were removed at 48 hours posttrauma ; Riluzole group of seven rats in which 1 mg/kg riluzole (Rilutek 50 mg Sanofi Saglik Urunleri L.T.D. Sti) was administirated intraperitoneally and 1 cm injured spinal cord samples and blood samples were removed at 48 hours posttrauma ;Trimetazidine and Riluzole group of seven rats in which 3mg/kg of trimetazidine (Sitorel 20 mg Mustafa Nevzat Ilac Sanayii A.S.) and 1 mg/kg riluzole (Rilutek 50 mg Sanofi Saglik Urunleri L.T.D. Sti) was administirated intraperitoneally and 1 cm injured spinal cord samples and blood samples were removed at 48 hours posttrauma.

Blood samples obtained from all groups were centrifuged at 3000 cycle in 10 minutes and plasma part was taken in to 2 epandorf tube. Samples were stored in -80 *C. Total Antioxidan Status (TAS) and Total Oxidan Status (TOS) levels were measured in the serum by the method which was explained by Erel (12, 13). Malondialdehid levels were measured by UV spectrofotometric method.

Spinal cord samples were fixed in a %10 formaline solution and observed by a pathologist who did not know the groups, treatments and the results of neurological evaluation of the rats. They observed the samples in 5 pm sections with Hematoksilen Eozin (H-E) paint. Abnormal findings and injuries of the cord were edited with the score system of Malinovsky (table 1) (14).

Table 1: Histopathological Scoring (14)

Grade 0	There is no abnormall cell and change
Grade 1	Mild hemorrhage and glial cell changes
Grade 2	Severe hemorrhage and changes with licefaction necrosis in the glial cells Severe hemorrhage and sponging associated with glial cell proliferation and liquefaction necrosis

Statistical Analysis

Statistical significance between experimental groups was defined using Kruskal-Wallis analysis of variance and the Mann-Whitney U test.

RESULTS

Biochemical Findings

Table 2 shows the levels of TAS , TOS and MDA measured in the blood. The mean level of TAS in control group was 1.02 mmol/L (±0,24 mmol/L). Metilprednisolone treatment significantly decreased the TAS levels.TAS levels in the Metilprednisolone performed group was 0,79 mmol/L (±0,14 mmol/L). Trimetazidine and Riluzole combine treatment shows similar decrease in the TAS levels like Metilprednisolone. TAS levels in the Trimetazidine and Riluzole combine performed group was 0,78 mmol/L (±0,09 mmol/L).

TOS levels were 29.14 mmol/L (±9,43 mmol/L). That means trauma increased the Tos levels . And in the table we saw that there is a significantly decrease in Trimetazidine and Riluzole combine performed group. Tos levels was 16,4 mmol/L (±2,12 mmol/L).

There was not a significant difference in the MDA levels between the groups . İn the control group MDA levels was 0,14mmol/L (±0,03mmol/L).

Table 2. Comparison of TAS, TOS and MDA levels between the groups.

	Ortalama±Standart Sapma		
	TAS	TOS	MDA
Travma	1,02±0,24	29,14±9,43	0,14±0,03
Metilprednizolon	0,79±0,14	24,24±6,80	0,25±0,16
Sham	1,00±0,10	47,61±23,08	0,17±0,09
Riluzole	0,82±0,08	23,14±5,97	0,21±0,11
Trimetazidine+Riluzole	0,78±0,09	16,40±2,12	0,29±0,24
Trimetazidine	0,82±0,08	31,86±20,88	0,24±0,20
p*	**0,001**	**<0,001**	0,257
*Kruskal Wallis Test			

PATHOLOGİCAL FİNDİNGS

In the table 3-9 pathological findings are shown as Malinovsky scoring data.

Table 3. Histopathological findings in all groups

	-	+	++	+++
Hemorrhage	32	6	4	
Licefaction	6	11	19	6
PNL	19	16	5	2
Lymphocyte	41	1		
Edema	7	27	8	

Table 4. Histopathological findings in Trauma group

	-	+	++	+++
Hemorrhage	4	1	2	
Licefaction	2		5	
PNL	4	3		
Lymphocyte	7			
Edema		5	2	

Table 5. Histopathological findings in metilprednisolone group

	-	+	++	+++
Hemorrhage	3	4		
Licefaction		1	2	4
PNL	2	5		
Lymphocyte	7			
Edema		7		

Table 6. Histopathological findings in the Sham group

	-	+	++	+++
Hemorrhage	7			
Licefaction		3	4	
PNL	4	3		
Lymphocyte	7			
Edema	2	5		

Table 7. Histopathological findings in Riluzole group

	-	+	++	+++
Hemorrhage	7			
Licefaction	1	4	2	
PNL	3	4		
Lymphocyte	7			
Edema	5	2		

Table 8. Histopathological findings in Trimetazidine and Riluzole combine group

	-	+	++	+++
Hemorrhage	5	2		
Licefaction			5	2
PNL	1	1	3	2
Lymphocyte	6	1		
Edema		2	5	

Table 9. Histopathological findings in Trimetazidine group

	-	+	++	+++
Hemorrhage	6	1		
Licefaction	3	3	1	
PNL	5	2		
Lymphocyte	7			
Edema		6	1	

Forming licefaction necrosis is decreasing in usage of metilprednisolone otherwise neither riluzole nor trimetazidine decrease forming licefaction necrosis.

Neurological Examination

All rats underwent neurological examinations before trauma, postoperative and 48 hours postoperatively and were evaluated according to Tarlov scoring system (15). Before the procedure, the neurological examination score of all rats was 4 according to Tarlov and the distributions according to groups 48 hours after surgery were summarized in the table 10.

Table 10. Neurological Examination Distributions in Groups After Trauma

		Neurological examinations				
		0	**1**	**2**	**3**	**4**
Grup	Trauma	4	3	0	0	0
	Metilprednizolon	2	4	1	0	0
	Sham	0	0	1	4	2
	Riluzole	5	2	0	0	0
	Trimetazidine+Riluzole	3	4	0	0	0
	Trimetazidine	5	2	0	0	0
		19	15	2	4	2

As shown in the table, there was no significant difference between the methylprednisolone and trimetazidine and riluzole combination groups in terms of neurological examination.

Discussion

The incidence of spinal cord injury due to spinal cord trauma is approximately 7500 to 10,000 per year, and in every 16 minutes new spinal injury occurs. In addition, examinations revealed that spinal cord trauma was most frequently seen in young people between the ages of 16 and 30 years (1, 2 , 16) . spinal cord trauma, is a serious disease that affects state policies and health system expenditures due to its socioeconomic effects as well as individual and social aspects.

The mechanism of damage occurring in spinal cord trauma occurs physiopathologically in two processes. The first is the primary injury that causes spinal cord damage by disrupting tissue integrity and blood supply from small bleeding foci at the time of the event to full-thickness incisions. Taking protective measures at this stage, and applying the spine and spinal cord protection measures in the transportation process from the patient's trauma to the hospital will be enough to prevent damage or deepening of the spinal cord. The other physiopathological stage is the secondary injury stage which occurs as a result of the physiopathological changes resulting from the primary injury. Secondary injury is caused by increased intracellular neuronal calcium content, increased intracellular free radical formation, increased lipid peroxidation, neurogenic shock, local vascular damage, biochemical disorders, electrolyte imbalance, edema, energy metabolism disorder and

ultimately apoptosis as a result of excitotoxicity and ischemia. Therefore, clinical studies focus more on secondary injury (17, 18).

Methylprednisolone has been shown to limit secondary tissue damage resulting from spinal cord trauma by reducing lipid peroxidation, regulating intracellular acid base balance, providing extracellular calcium balance and increasing Na K ATPase activity, reducing water and salt retention and preventing potassium loss. In addition, high-dose methylprednisolone treatment has been reported to increase spinal cord blood flow and neural tissue perfusion. The importance of methylprednisolone in the treatment of acute spinal cord injury in spinal cord trauma has been demonstrated in NASCIS II and NASCIS III studies with multicenter and large studies (19, 20, 21, 22).

Topsakal C et al. In a spinal cord trauma model study, the efficacy of methylprednisolone was investigated and it was reported to be effective in spinal cord trauma due to its inhibition of lipid peroxidation and neuroprotective effect(23). In a study comparing the efficacy of methylprednisolone and tadafil in a spinal cord trauma performed by Serarslan et al., It was shown that MDA levels increased after spinal cord trauma and decreased after methylprednisolone treatment(24). In our study, it was found that elevated MDA levels increased after spinal cord trauma and there was no significant difference between methylprednisolone, trimetazidine and riluzole groups after treatment.

Riluzole is an anticonvulsant drug with neuroprotective properties. It mainly acts with Na channel blockage and due to its neuroprotective efficacy, it is frequently used in spinal cord injuries and some neurodegenerative diseases(25). It has been observed that by blocking the voltage-dependent Na channels, inhibits the release of glutamate from the presynaptic area and thereby increases the healing by decreasing the severity of the lesions developing as a result of spinal cord injury, decreasing axonal degeneration and increasing axonal regeneration (26, 27, 28, 29) .

Trimetazidine, on the other hand, is a cardiac anti-ischemic agent which has been shown to be effective in increasing ATP production, reducing lipid peroxidation in the membrane and limiting free radical formation(30, 31). Trimetazidine, which also has antioxidant activity, has also been shown to have a neuroprotective effect due to recent studies(32, 33).

In this study, we investigated the effect of Riluzole and Trimetazidine , separately and in combination , on spinal cord injury due to spinal cord trauma with histopathological, biochemical and motor function evaluation and compared with high dose methylprednisolone treatment group.

We performed motor function evaluation according to Tarlov scale. We evaluated the results of rats who underwent motor function evaluation before and 48 hours after surgery, on Tarlov scale. As a result, it was seen that motor function evaluation in the group where trimetazidine and riluzole were used together was close to methylprednisolone group, but there was no significant effect in groups where riluzole and trimetazidine were given separately.

Although the scoring criteria determined by Malinovsky et al were used in the histopathological evaluation, necrosis development was significantly reduced in the high-dose methylprednisolone group, but no significant difference was observed in the groups given separately and in combination with riluzole and trimetazidine (14).

In biochemical evaluation, Kanter et al. Showed an increase in MDA levels in spinal cord tissue in spinal cord injury (34). In our study, an increase in MDA was detected in patients with spinal cord trauma. However, although there was no significant difference between malondialdehyde values after treatment, total antioxidant status was found to be close to those of trimetazidine and riluzole combined with high doses of methylprednisolone. In the Total Oxidant Status assessment, the values of the group given high dose methylprednisolone and the group given trimetazidine and riluzole and the groups in which riluzole was given alone were found to be close to each other.

In the light of this information, the effects and mechanisms of action of methylprednisolone on secondary injury, which have been proven in multicentre and large-scale studies which have been effective in the treatment of spinal cord injury due to spinal cord trauma, have been shown. Studies on the dose and duration of administration of methylprednisolone and the search for an alternative to methylprednisolone and / or combination of methylprednisolone in the ongoing studies are continuing. Although there are studies in the literature on the neuroprotective efficacy of trimetazidine and riluzole, it was not found that they were combined and compared with methylprednisolone efficiacy as motor function, biochemical values and histopathology.

In the light of the data of this study , in addition to high-dose methylprednisolone treatment which has an important place in the current treatment scheme in the treatment of spinal cord trauma , combined use of trimetazidine and riluzole is thought to be beneficial both histopathologically and biochemically. Although there was no significant effect on trimetazidine and riluzole in separate use, it is clear that it would be beneficial to extend the study over a longer period of time and using more extensive data.

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