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The Use of Methylprednisolone, Vitamin E and Their Combination in Acute Spinal Cord Injury: An Experimental Study

Akut Omurilik Yaralanmasında Vitamin E, Metilprednizolon ve Kombinasyonunun Kullanımı: Deneysel Çalışma

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ABSTRACT

OBJECTIVE: The effects of methyprednisolone (MP) and Vitamin E (Vit E) combination treatment was investigated in an experimental spinal cord injury model in rats.

METHOD: Thirty-one rats were randomly divided into the five following groups: control group, MP group, Vit E group, MP+Vit E group and sham operated group. A spinal cord injury was produced in the rats by using a compression injury model at the T8 vertebra level for 10 minutes. MP was injected as a 30 mg/kg IV bolus, 1 hour after the injury, followed by an infusion of 5.4 mg/kg for 23 hours. Vit E was administered as a 30 mg/kg IV bolus at the posttraumatic 1st, 7th , 13th and 19th hours. The sham group underwent laminectomy without spinal cord compression and did not receive medication. The animals were sacrificed at the posttraumatic 48th hour and histopathological examination was performed in a blinded fashion for the following criteria: hemorrhage, necrosis, edema, microcyst, microglia proliferation and PMNL infiltration.

RESULTS: The pathology evaluation of the groups revealed that the MP+Vit E combination treatment impeded the progress of edema/microcyst formation, microglia proliferation, and necrosis.

CONCLUSION: Vit E, when combined with MP for spinal cord injury treatment, augments the effect of MP probably due to its antioxidant effects.

KEY WORDS: Lipid peroxidation, Methylprednisolone, Spinal cord injury, Vitamin E.

ÖZ

AMAÇ: Sıçanlarda deneysel omurilik yaralanması modelinde metilprednizolon (MP) ve Vitamin E (Vit E) kombine tedavisinin etkilerini araştırmak.

YÖNTEM: Otuz bir Wistar sıçan kontrol grubu, MP grubu, Vit E grubu, MP+Vit E grubu ve Sham grubu olmak üzere 5 farklı gruba ayrıldı. Sıçanların omuriliğine T8 omuru düzeyinde 10 dakikalık kompresyon hasarı modeli uygulandı. MP travmadan 1 saat sonra 30 mg/kg IV bolus olarak ve sonraki 23 saat boyunca 5.4 mg/kg'dan infüzyon olarak verildi. Vit E 30 mg/kg IV bolus olarak travma sonrası 1., 7., 13. ve 19. saatlerde verildi.

Sham grubuna sadece laminektomi yapılıp herhangi bir kompresyon veya medikasyon uygulanmadı. Hayvanlar travma sonrası 48. saatte sakrifiye edildi ve şu kriterlere göre histopatolojik olarak kör inceleme yapıldı: hemoraji, nekroz, ödem, mikrokist, mikroglial hücre artışı ve polimorfonuklear lökosit infiltrasyonu.

BULGULAR: Grupların histopatolojik değerlendirilmesi sonucunda MP ve Vit E'nin kombine tedavisinin ödem, mikrokist oluşumu, mikroglial hücre artışı ve nekrozu diğer gruplara göre daha iyi oranda azalttığı bulundu.

SONUÇ: Vit E omurilik hasarı tedavisinde, MP ile kombine uygulandığında, muhtemelen antioksidan etkilerinin yardımı ile MP'nin etkilerini artırmaktadır.

ANAHTAR SÖZCÜKLER : Lipid peroksidasyonu, Metilprednizolon, Omurilik hasarı, Vitamin E

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INTRODUCTION

Acute spinal cord injury (SCI) resulting in various degrees of neurological deficit is a devastating condition, both physiologically and psychologically, and the present methods of treatment offer limited hope for restoration of neurological function. Current opinion is that acute SCI is a two-step process with primary and secondary mechanisms. The former involves the mechanical insult and subsequent compression of the spinal cord, initiating a cascade of biochemical and cellular processes known as the secondary injury mechanisms (7,8). Briefly, secondary injury mechanisms involve both vascular and neuronal responses including hemorrhage, loss of microcirculation, ischemia, decreased intracellular ATP production, lactic acidosis, influx of calcium ions, increased extracellular potassium, production of free oxygen radicals, membrane hydrolysis by lipid peroxidation (LP), prostaglandin and leukotriene generation and edema formation (6,7,9,30). Among these, LP is thought to be the main secondary mechanism after acute injury (16,32,33).

There is currently considerable confusion and controversy regarding the treatment of acute SCI. During the last two decades, numerous studies have been made to determine the 'standard' treatment protocol. The therapeutic effect of a variety of agents including MP (36,6,7), gangliosides (30), naloxone (37), trilazad mesylate (9), nimodipine (14), ginkgo biloba (24), activated protein C (17), thyrotropine releasing hormone (24), prostacyclin (9), Vit E (29), cycloheximide (27) and gabexate mesylate (34) has been investigated in both clinical and experimental studies. Among these agents, the use of MP infusion following acute SCI was widely approved mainly following the results of the National Acute Spinal Cord Injury Studies [NASCIS I, II, III] (5,6,8). The NASCIS studies confirmed that high doses of MP improve neurological recovery if administered within 8 hours of injury. Up to now, the most likely explanation for the protective effect of MP's in SCI is that MP suppresses membrane breakdown by inhibiting LP (10,22,34).

Vit E (alpha-tocopherol) is a fat-soluble antioxidant, participating in reduction reactions that aim to remove the free radicals in the body. In addition to its antioxidant effect, Vit E enhances the regional microcirculation and its protection against LP is extremely important for the functional integrity of all biologic membranes (5,13,15).

The outcome of MP and Vit E therapy has been investigated individually in numerous experimental models of SCI. However, no study has yet assessed the effects of a combination treatment (MP+Vit E) in a rat model of spinal cord compression injury. Our purpose was to evaluate the results based on histopathological criteria.

MATERIAL AND METHODS

The experimental procedure was approved by the ethical committee for animal experiments of our university. All of the procedures were done according to the accepted standards of the Guide for Care and Use of Laboratory Animals. The study was carried out on thirty-one male Wistar rats, weighing 260-300 grams. The rats were randomly divided into the following five groups: control group (n=6); MP group (n=8); Vit E group (n=6); MP+Vit E group (n=5); and sham operated group (n=6).

The rats were anesthetized using halothane; they were then intubated endotracheally and ventilated with room air. Femoral arterial and venous catheters were placed to monitor the blood pressure and arterial blood gas values, and administer fluids and drugs. The body temperature was monitored by using rectal probes and maintained at 36,5 - 37°C by heating pads.

The spinal cords were carefully exposed following microsurgical removal of the vertebral lamina at the T8 level. To injure the cord, the compression injury model described by Morino et al. (28) was used. Briefly, an iron stick with a soft, rounded conical silicone on its tip, weighing 20 g in total, was gently and slowly placed on the exposed spinal cord extradurally and kept for ten minutes.

The doses of MP and Vit E were determined based on previous studies (12,26,29). MP was injected as 30 mg/kg IV bolus,1 hour after the injury, followed by an infusion of 5.4 mg/kg for 23 hours. Vit E dose was also administered via the intravenous route as a 30 mg/kg IV bolus at the posttraumatic 1st, 7th, 13th and 19th hours. The sham group underwent laminectomy without trauma and did not receive any medication.

The animals were sacrificed at the post-traumatic 48th hour. The spinal cord at the 8th thoracic vertebra level was immediately and atraumatically removed for histopathological examination. The specimens were fixed with formalin (10%). After fixation, the cords were embedded in paraffin and microtome subserial sections of 4μ thickness were

prepared. The slices were then stained with Hematoxylin and Eosin (H-E) and examined in a blinded fashion under the light microscope. The traumatic injury was graded histopathologically as negative (no change); +1 (mild pathological changes); +2 (moderate pathological changes) or +3 (severe pathological changes).

STATISTICAL ANALYSIS

The unpaired Student t-test was used for statistical analysis. $P<0,05$ was considered significant.

RESULTS

The sequential pathological changes after acute injury to the spinal cord include hemorrhage, edema, necrosis, microcyst formation and infarction. In the pathological specimens of the control group, these changes were clearly identified (Figure 1). The degree of microcyst formation for the MP group and Vit E group were moderate and mild, respectively. Examination of neither the MP group (Figure 2) nor the Vit E group (Figure 3) revealed apparent changes except for a moderate decrease in the edema and microcyst formation when compared to the control group ($P=0,01$). The interesting point is that striking changes were identified on the specimens of the MP+Vit E group (Figure 4). There was a marked decrease in the amount of hemorrhage, necrosis and microcyst formation when compared to the other groups ($P=0,009$). In addition, the process of edema formation and microglia proliferation was shown to be mild or negative, supporting the hypothesis that the combination therapy of MP and Vit E may augment each other's effect at least in terms of histopathological criteria. The results are summarized in Tables I and II.

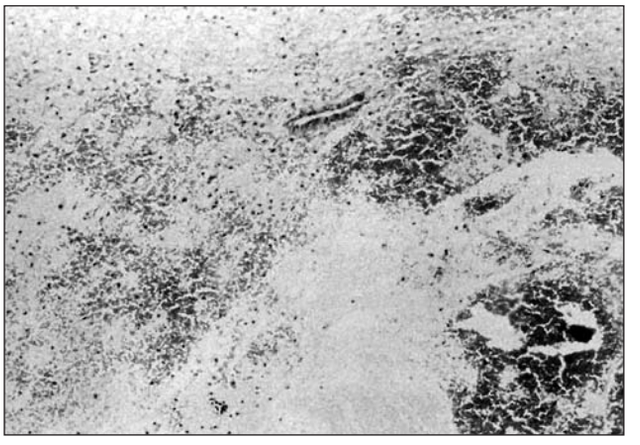

Figure 2: MP group: Hemorrhage and microcyst formation. Mild edema and necrosis are noted (HEx100).

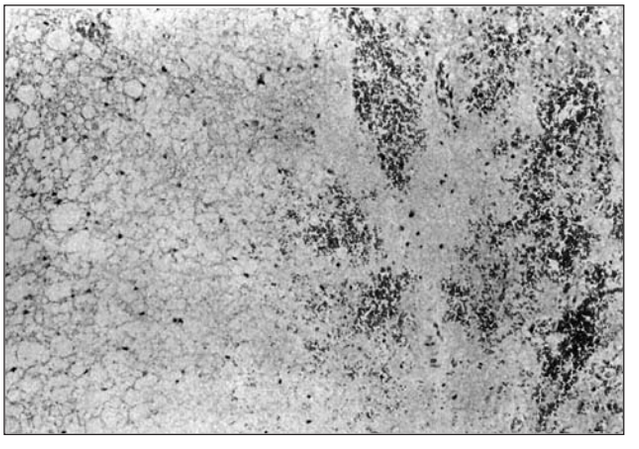

Figure 3: Vit E group: Hemorrhage and microcyst formation (HEx100).

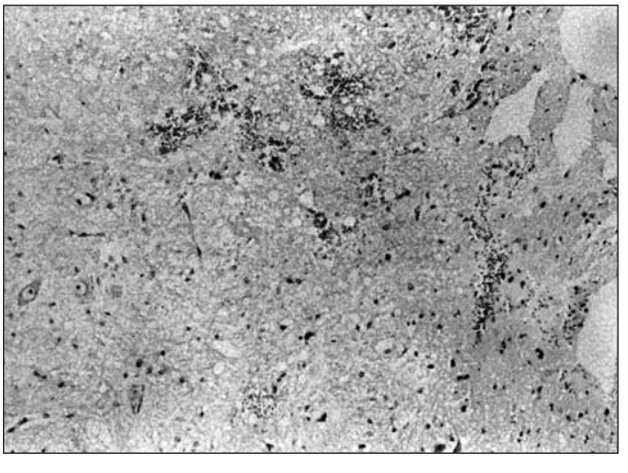

Figure 1: Control group: Severe hemorrhage, moderate edema and microcyst formations (HEx100).

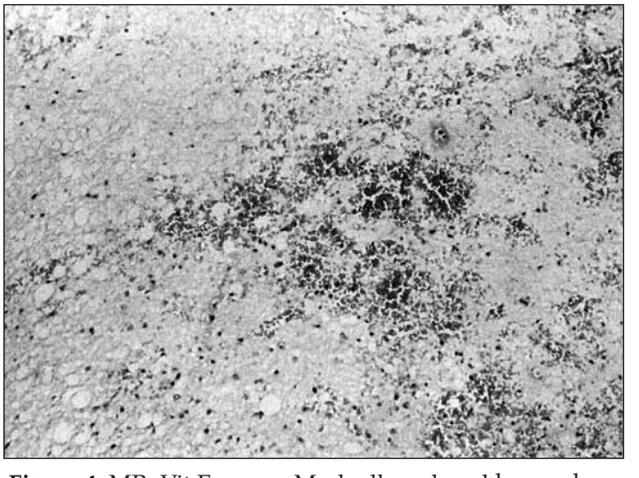

Figure 4: MP+Vit E group: Markedly reduced hemorrhage and necrosis. There is mild microcyst formation (HEx100).

Table I. Summary of histopathological findings of the rat groups.

Groups	Hemorrhage	Necrosis	Edema	Microcyst Proliferation	Microglia	PNL infiltration of meninges
Sham n = 6						
Control	Negative	Negative	Negative	Negative	Negative	Negative
1	3+	1+	2+	2+	1+	1+
2	3+	1+	1+	2+	Negative	1+
3	3+	1+	2+	2+	1+	2+
4	1+	Negative	1+	1+	Negative	Negative
5	3+	1+	2+	2+	1+	2+
6	1+	Negative	1+	1+	1+	1+
MP						
1	3+	1+	1+	2+	1+	Negative
2	2+	1+	1+	1+	Negative	Negative
3	3+	1+	1+	3+	Negative	1+
4	2+	1+	1+	3+	1+	1+
5	1+	Negative	Negative	2+	1+	Negative
6	2+	1+	1+	1+	1+	1+
7	Negative	Negative	Negative	Negative	Negative	1+
8	2+	1+	Negative	1+	Negative	1+
Vit E						
1	1+	Negative	Negative	1+	2+	1+
2	3+	1+	1+	1+	1+	2+
3	3+	1+	1+	1+	1+	Negative
4	3+	1+	1+	2+	1+	3+
5	2+	Negative	Negative	1+	1+	Negative
6	3+	1+	Negative	1+	1+	Negative
MP+Vit E						
1	1+	Negative	Negative	1+	Negative	Negative
2	1+	Negative	Negative	1+	Negative	Negative
3	1+	1+	1+	1+	1+	Negative
4	1+	Negative	1+	1+	1+	1+
5	2+	Negative	Negative	2+	Negative	2+

MP: methylprednisolone; PNL: polymorphonuclear leucocytes; T: trauma; 1+: mild changes; 2+: moderate changes; +3: severe changes

Table II. The mean values of the histopathological criteria scores among different groups.

	Control	MP	MP+Vit E
Hemorrhage	2.33 ± 1.03	1.88 ± 0.99	1.20 ± 1.44
Necrosis	0.66 ± 0.51	0.75 ± 0.46	0.20 ± 0.44
Edema	1.50 ± 0.54	0.62 ± 0.51	0.40 ± 0.54
Microcyst	1.67 ± 0.51	1.62 ± 1.06	1.20 ± 0.44
Microglia	0.66 ± 0.51	0.50 ± 0.53	0.40 ± 0.54
PNL infiltration	1.17 ± 0.75	0.62 ± 0.51	0.60 ± 0.89

DISCUSSION

Despite intensive preventive efforts and modern surgical techniques, the treatment of acute SCI remains obscure and SCI continues to be a significant health problem with high morbidity and mortality rates. For this reason, many efforts have been made to understand the pathophysiological mechanisms, which may help to expose appropriate treatment in SCI. After the primary injury caused by mechanical insult, secondary injury mechanisms that lead step by step to neuronal death appear (7,8). Nearly all authors believe that the secondary mechanisms may be prevented by some treatment strategies and a variety of agents have been developed in experimental SCI studies (6,7,9,14,30,31,36,37). In patients with central nervous system injury, LP has been believed to be a major culprit for permanent neurological dysfunction (16,23). Therefore, treatment protocols have targeted LP and MP is the most popular regimen used following SCI among the protocols proposed. Use of MP has became popular especially after the results of the NASCIS II and III studies were reported (6,8) and it is accepted that the primary action of MP is the inhibition of posttraumatic LP and to protect the structural and functional integrity of biological membranes (6,8). However, recent reports have re-evaluated the results of NASCIS and other studies and examined the effect of steroids on acute, nonpenetrating SCI and stated that MP as an "inappropriate standard of care" in acute SCI (17-19).

There is also a natural line of defense mechanisms including antioxidant enzymes and vitamins. Vit E is one such vitamin that has neuroprotective effects due to its antioxidant features (4,20,21). Studies have shown that Vit E administration in the setting of acute SCI significantly decreases LP and edema formation, and Vit E-deficient rats showed lower recoveries in the amplitude and latency of spinal cord evoked potentials (2,20,35). Pretreatment with Vit E reduced post-injury tissue necrosis and paralysis (2). Based on the studies with Vit E, it is strongly believed that Vit E has a neuroprotective effect and decreases the detrimental consequences of LP after acute SCI.

It is the beyond the scope of this paper to explain the secondary mechanisms following acute SCI and the mechanisms by which MP and Vit E prevent LP as these are explained in details in other articles. Our aim is to show briefly the results of using MP and Vit E after acute SCI on the basis of histopathological evidence.

In this study, we investigated six pathological changes seen in neuronal tissues following central nervous system injuries. These are as follows: hemorrhage, necrosis, edema formation, microcyst formation, microglia proliferation, and polymorphonuclear (PNL) infiltration of the meninges. Table 1 summarizes the pathological changes noted on the specimens following the trauma. As can be expected, no pathological changes were noted in the sham group and the changes we evaluated were marked in the majority of the specimens belonging to the control (trauma only) group. Regarding the pathological changes, no major differences were found between the control group and MP group except for a mild decrease in edema formation in the latter one. This fact was proven statistically as the comparison of the control group and MP group revealed insignificant values except for the decrease in edema formation in the latter group (P=0,01). The most striking findings were noted in the specimens belonging to MP+Vit E group. A significant decrease in the amount of hemorrhage and microcyst formation was seen and necrosis, microglia proliferation, and PNL infiltration of the meninges were also less compared to the other groups. These findings however were statistically insignificant except for the marked decrease in edema formation (P=0,009).

The mean values of the pathological criteria for the groups are summarized in Table 2. As can be seen, the overall values tend to decrease in the MP group and this decrease is much more significant when Vit E is added (MP+Vit E group). We therefore believe the statistically insignificant results are due to the small animal population used. Our results support the results of the studies that re-evaluated the results of NASCIS and others evaluating the effects of MP in the setting of SCI (5,6,8,16-19).

The neuroprotective effects of the combination treatment (MP+Vit E) has been shown in ischemic brain damage (11) and to our knowledge this is the first study that shows the neuroprotective effects of such combination treatment in acute SCI. An antioxidant, Vit E has been shown to have an inhibitory effect on LP after SCI (1,3,25). It has also been demonstrated that amount of increase of lipid peroxides are directly proportional to an increase in

the compression weight and a decrease in the level of Vit E intake in the setting of acute SCI in rats (35).

The degree of functional recovery, which was not evaluated in this study, might provide a better evaluation of the results, but this preliminary study was intended to investigate the histopathological changes. We believe our results can be helpful for planning further future studies.

Taken together, the results demonstrate that Vit E is useful tool for decreasing LP following acute spinal cord trauma and that MP is more effective when combined with an antioxidant agent. Treatment strategies should therefore include an antioxidant agent in addition to MP or other drugs following acute SCI.

CONCLUSION

Although there is much debate on the use of MP in acute SCI, physicians are commonly using it all over the world. However, there is growing body of evidence that antioxidant agents have more prominent neuroprotective effects than MP and that neuronal death will be prevented in the ischemic brain when they are combined, In addition, the beneficial effects of antioxidants and especially Vit E has been demonstrated in experimental studies. This preliminary study showed that MP and/or Vit E alone has a mild beneficial effect but the combination produced an additive effect for the prevention of the pathological changes following acute SCI. Therefore, we suggest the use of MP in combination with Vit E in the setting of acute SCI. Further studies should be designed in a large animal population in order to figure out the exact mechanisms that might explain how MP plus Vit E decreases the catastrophic consequences of acute SCI.

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