Eur Spine J (2004) 13 : 724–732
DOI 10.1007/s00586-003-0550-y

ORIGINAL ARTICLE

Suleyman R. Cayli
Ayhan Kocak
Ugur Yilmaz
Ayhan Tekiner
Mine Erbil
Cetin Ozturk
Kadir Batcioglu
Saim Yologlu

Effect of combined treatment with melatonin and methylprednisolone on neurological recovery after experimental spinal cord injury

Received: 4 February 2002
Revised: 10 February 2003
Accepted: 21 February 2003
Published online: 1 July 2004

The number of references, figures of waveforms obtained from CSEP and MEP tests, and electron micrographs of the samples obtained from trauma and all treatment groups at the 10th day postinjury have been reduced in the printed version. The complete version of the article will be published in the web version of the Journal

S. R. Cayli (✉) · A. Kocak · A. Tekiner
Department of Neurosurgery,
İnönü University Medical Faculty,
PK 230 Malatya, Turkey
Tel.: +90-422-3410660/3210,
e-mail: srcayli@hotmail.com

U. Yilmaz
Department of Urology,
Neurourology-Neurophysiology Laboratory,
İnönü University Medical Faculty,
Malatya, Turkey

M. Erbil
Department of Anatomy,
Hacettepe University Medical Faculty,
Ankara, Turkey

C. Ozturk · K. Batcioglu
Department of Biochemistry,
İnönü University Medical Faculty,
Malatya, Turkey

S. Yologlu
Department of Biostatistics,
İnönü University Medical Faculty,
Malatya, Turkey

Abstract Spinal cord injury (SCI) results in the loss of function below the lesion. Secondary injury following the primary impact includes a number of biochemical and cellular alterations leading to tissue necrosis and cell death. Methylprednisolone (MP), by reducing edema and protecting the cell membrane against peroxidation, is the only pharmacological agent with a proven clinically beneficial effect on SCI. Melatonin, known as a free radical scavenger, has been shown to have an effect on lipid peroxidation following experimental SCI. The purpose of this study was to examine the effect of MP and melatonin on neurological, ultrastructural, and electrophysiological recovery. Female albino rats weighing 200–250 g were randomized into five groups of 18 rats each and six rats for the control group. Weight-drop trauma was performed for each group and a 30-mg/kg single dose of MP for rats in group 1, a 10-mg/kg single dose of melatonin for rats in group 2, and MP and melatonin in the same doses for rats in group 3 were administered immediately after trauma. The rats in group 4 were the vehicle group (treated with ethanol) and group 5 was the trauma group. The motor and somatosensory evoked potentials were recorded at the 4th hour, the 24th hour, and on the 10th day of the study for six rats in each group. Posttraumatic neurological recovery was recorded for 10 days using "motor function score" and inclined plane test. After electrophysiological study the rats were terminated for an analysis of lipid peroxidation level of the injured site of the spinal cord. Electron microscopic studies were performed to determine the effects of melatonin, MP, and the combined treatment with MP and melatonin on axons, neurons, myelin, nucleus, and intracytoplasmic edema. The groups treated with MP, melatonin, and a combination of both had significantly enhanced electrophysiological, biochemical, and neurological recovery and also showed better ultrastructural findings than the trauma and vehicle groups. Although combined treatment was significantly more effective on lipid peroxidation than melatonin or MP treatments alone, at the 10th day, neurobehavioral, electrophysiological, and ultrastructural recovery were at the same level. In conclusion, MP, melatonin, and MP and melatonin combined treatment modalities improved functional recovery at the same level. Future studies involving different doses of melatonin and different dose combinations with MP could promise better results since each drug has a different antioxidative mechanism of action.

Electronic Supplementary Material Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00586-003-0550-y

Keywords Spinal cord injury · Methylprednisolone · Melatonin · Evoked potentials · Lipid peroxidation

Introduction

The pathophysiology of acute spinal cord injury (SCI) is complex and two mechanisms can be distinguished as the primary and secondary damages [7]. The initial mechanical damage to the spinal cord, which includes both the kinetic impact to the spinal cord and the persistent compression of the cord by shifted bone fragments, is referred to as the primary damage [9]. Many pathological changes seen after spinal cord trauma are thus secondary to the initial impact and include edema, altered blood flow, and changes in microvascular permeability [26]. Pharmacological intervention in the acute phase of SCI aims to counteract secondary neurotoxic events or to interrupt the progression of this process. To explain this delayed tissue damage, numerous pathophysiological mechanisms have been postulated. Many studies have showed that one of the most important factors precipitating posttraumatic degeneration in the spinal cord is oxygen free radical-induced lipid peroxidation [2, 14].

To date, relatively little progress has been made in the treatment of SCI and related neurological impairments. High doses of methylprednisolone (MP) have a clinically proven beneficial effect on functional recovery after SCI in humans [4] and improve neurological recovery after experimental trauma to the spinal cord [3, 8]. The underlying mechanism is not fully understood, but experimental data point to protection against membrane peroxidation and edema [5]. Further research has shown that the high dose of MP required to inhibit lipid peroxidation also exerts a number of other actions on the injured spinal cord such as reduction in posttraumatic ischemic area [15, 27], neurofilament degradation [6], reversing intracellular calcium accumulation and preserving evoked potentials, and improving spinal cord blood flow [27].

The pineal hormone, melatonin, is a very effective antioxidant agent similar to MP [23, 24] and it has been shown to enter the cell and nucleus [21]. In previous studies, melatonin has been shown to protect against nucleic acid damage via an action involving generation of oxygen-based free radicals produced by the administration of the chemical carcinogen safrole and ionizing radiation [16, 24]. A few previous studies have shown the effectiveness of melatonin on lipid peroxidation in SCI [12, 18]. The purpose of the present study was to investigate neurobehavioral and ultrastructural recovery and evaluate the electrophysiological and biochemical responses to treatment of experimental SCI with single low dose melatonin, MP, and combined treatment of both agents.

Materials and methods

Adult female albino rats weighing 200–250 g were used in this study. Before surgery, all rats were tested and a normal motor function was found. The animals were anesthetized by an intraperitoneal injection of chloral hydrate (400 mg/kg) and pinned in the prone position. Under sterile surgical conditions and following a midline skin incision at T5–12 and paravertebral muscle dissection, spinous processes and laminar arcs of T7–10 were removed with the assistance of a surgical microscope. The dura was left intact. Weight-drop trauma modeling was performed for all the animals [1]. The animals were subjected to an impact of 50 g/cm to the dorsal surface of the spinal cord, rendering them severely wounded and paraplegic. After the trauma, the muscles and incision were sutured with 6-0 vicryl suture (Ethicon).

The rats were randomly allocated in six groups: a control (sham-operated) group of six rats in which only a laminectomy was performed, a trauma group, an MP group, a melatonin group, an MP and melatonin group, and a vehicle (ethanol) group, each group containing 18 rats. A single dose of MP (30 mg/kg; Mustafa Nevzat Ilac Sanayi A.S.) for the MP-treated group, a 10-mg/kg single dose of melatonin (Sigma Chemicals, St. Louis, MO, USA) for the melatonin-treated group, a 30-mg/kg single dose of MP and a 10-mg/kg single dose of melatonin for the MP and melatonin-treated group, and 0.2 ml vehicle (ethanol) for the vehicle-treated group was administered intraperitoneally immediately after trauma. Then, all the treated groups were separated into three groups at 4 h, 24 h, and 10 days following trauma. All rats in the 10 days group received gentamicin twice daily during the first 3 days as prophylaxis against urinary tract infection. Bladders were emptied manually twice a day during this period. In case of mortality in any group, additional rats were assigned to ensure a minimum of six rats for each study subgroup.

The motor and cortical somatosensory evoked potentials (MEP and CSEP) were obtained for the MP, melatonin, MP–melatonin, ethanol, and trauma groups at the 4th hour, the 24th hour, and on the 10th day after trauma. Posttraumatic neurologic recovery was recorded for 10 days for the rats in the 10 days group. After electrophysiological testing, all the animals were killed and 1-cm spinal cord samples were removed for determination of lipid peroxidation and electron microscopic study.

Electrophysiology evaluation

Cortical somatosensory evoked potential measurements

All the tests were performed with a two-channeled Medelec Synergy EMG/EP machine (Oxford Instruments, London). The left sciatic nerve was isolated and stimulated with a pair of hook electrodes, a proximal anode and a distal cathode, in order to obtain CSEP. The duration of stimuli was 0.2 ms at 3 pps and the stimulus amplitude of each test was at a threshold sufficient for moving the ipsilateral foot. Two silver screw electrodes were placed epidurally over the somatosensory cortex and on the maxillar region in contact with the hard palate as active and reference electrodes, respectively. A silver disc electrode was placed on the right gluteal region for grounding. The tests were performed twice each time and in each test 400 CSEP were averaged in an analysis time of 50 ms. The frequency filters were 1–100 Hz. When there were significant differences between the two successive tests, the procedure was repeated three or four times to evaluate test-to-test variability. The sensitivity of waveforms was arranged between 5 and 20 μV according to the amplitude of obtained cortical potential. The latency of CSEP was measured from the onset of the first negative deflection from the baseline. The amplitudes were measured from the distance between the first negative and positive peaks.

Motor evoked potentials

Two silver screw electrodes were placed epidurally over the right motor cortex area and on the hard palate as active and reference electrodes, respectively, to achieve transcortical stimulation by applying square wave pulses of 0.1 ms at 3 pps. The motor responses

were recorded from two pairs of silver hook electrodes, as active and reference electrodes, placed 1 cm apart on the left sciatic nerve. A silver disc electrode was placed on the right gluteal region for grounding. Sciatic nerve was manipulated carefully while paying attention not to injure the nerve. The motor responses were recorded at frequency filters of 10 and 500 Hz and 200 MEP were averaged with a sweep of 50 ms. The tests were performed twice each time and when there were significant differences between the two successive tests, the procedure was repeated three or four times to evaluate test-to-test variability. The sensitivity of waveforms was arranged between 5–20 μV according to the amplitude of obtained motor response. The latency of MEP was measured from the onset of the first positive or negative deflection from the baseline and the amplitude was measured between the positive and negative peaks of averaged waveforms. The waveforms obtained from the sciatic nerve represented the motor conduction velocity rather than motor unit potentials as measured from the innervated muscle.

Behavioral assessment

For behavioral testing, we used a "motor function scale" [10] that is a slight modification of the "motor score" proposed by Gale et al. [13]. The animals were allowed to move freely in an open field (0.7×0.9 m) and were observed for at least 1 min. Movements in the hip, knee, and ankle joints were recorded.

In a second behavioral test, an inclined plane technique was used. Rivlin and Tator's angle board test consisted of measuring the maximum angle at which an animal can support its weight on an inclined plane measured in degrees from 0 to 90 [25]. The animals were placed transversely on the inclined plane and the highest angle a rat can maintain for 5 s was recorded and described as the "capacity angle" for that animal.

Ultrastructural examination

One millimeter of cross-sectional gray and white matter at the epicenter of the spinal cord lesion was removed for ultrastructural examination. The spinal cord samples were placed in 2.5% glutaraldehyde for 24 h for fixation. The tissue was postfixed with OsO_4, dehydrated in a graded series of alcohol, and then embedded in Araldite CY212. Semithin sections (2 μm) were stained with toluidine blue and observed by light microscopy. The thin (60- to 90-nm) sections were stained with uranyl acetate–lead citrate, examined on an electron microscope (JEM 1200EX; Jeol, Tokyo, Japan), and photographed. In the observation, each sample was evaluated according to a grading system for quantitative evaluation, which established different tissue samples and SCI [17].

Lipid peroxidation

The exposed spinal cord segments were removed in all groups of rats at 4 h, 24 h, and 10 days after injury. The samples were immediately frozen and stored in a –20°C freezer for assays of malondialdehyde (MDA). The levels of lipid peroxides in traumatized spinal cord tissue were measured as thiobarbituric acid-reactive material and determined using the method of Mihara and Uchiyama [22]. MDA has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535 nm. The assay procedure for lipid peroxide in spinal cord tissue was set up as follows. Tissues were homogenized in 10 vol (w/v) cold 1.5% KCl. Half a millimeter (0.5 ml) of homogenate was mixed with 3 ml 1% H_3PO_4 and 1 ml 0.6% thiobarbituric acid. The mixture was then heated in boiling water for 60 min. After cooling, the color was extracted into 4 ml n-butanol and the absorbance was recorded at 535 and 520 nm. Using tetramethoxypropane as the standard, tissue lipid peroxidate levels were calculated as nanomoles per gram of wet tissue.

Statistical analysis

All results were presented as mean ± SD. Statistical significance between groups was defined using Kruskal-Wallis analysis of variance and the Mann-Whitney *U*-test. The statistical difference between postinjury time for all groups was evaluated using the Friedman analysis of variance. A *P* value less than 0.05 was considered statistically significant.

Results

Electrophysiological evaluation

The 4th-hour CSEP and MEP recordings were nondetectable in trauma and ethanol groups. The ethanol group revealed no significant difference in the mean amplitudes and latencies of CSEP and MEP when compared to the trauma group in the first and tenth postoperative days, respectively ($P>0.05$ for each). The postoperative first-day CSEP and MEP measurements revealed significantly longer mean latencies and lower amplitudes in the trauma group in comparison with MP, melatonin, and MP+melatonin groups ($P<0.05$ for each). There was no significant difference in the mean amplitude and latency measurements between the MP, melatonin, and MP+melatonin groups at the tenth postoperative day in both CSEP and MEP studies ($P>0.05$ for each). When the groups were compared with each other at the 10th day, ethanol and trauma groups had significantly longer mean latencies and lower mean amplitudes of CSEP and MEP tests than those of the MP, melatonin, and MP+melatonin groups ($P<0.05$ for each; Fig. 1a–d).

Evaluation of the motor function

The results of the "motor function score" evaluation are presented as mean value ± SD in Fig. 2. Trauma caused a significant change in motor function score as compared to the control group values. A significant gradual recovery rate was observed in the rats treated with MP, melatonin, and MP+melatonin ($P<0.05$). Statistical analysis revealed similar difference rates between all groups except for the trauma group ($P>0.05$).

The results of the inclined plane evaluations are presented as mean values ± SD in Fig. 3. Trauma produced a highly significant decrease in angle score which, then, gradually recovered over a period of 10 days in the rats treated with MP, melatonin, and MP+melatonin ($P<0.05$). There was no significant increase in angle score for the rats in the trauma group ($P>0.05$). In all medically treated rats, the motor function score and the capacity on the inclined angle board improved gradually during the obser-

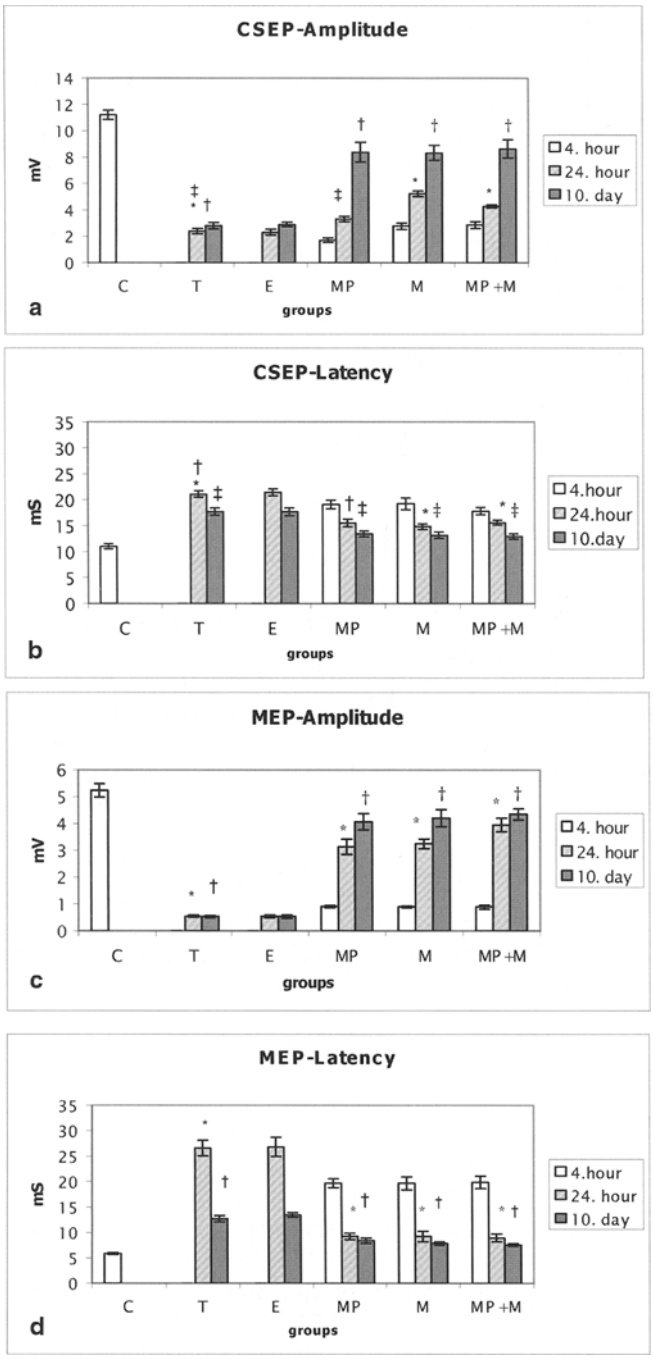

Fig. 1a–d Graphs showing the mean values of cortical somatosensory evoked potential (*CSEP*) amplitude (**a**; ‡ P=0.016, † P=0.004, * P=0.004) and latency (**b**; † P=0.004, * P=0.002, ‡ P=0.002) levels and motor evoked potential (*MEP*) amplitude (**c**; * P=0.004, † P=0.004) and latency (**d**; * P=0.004, † P=0.004). *C* Control group, *T* trauma group, *E* vehicle (ethanol) group, *MP* methylprednisolone group, *M* melatonin group, *MP+M* methylprednisolone+melatonin group

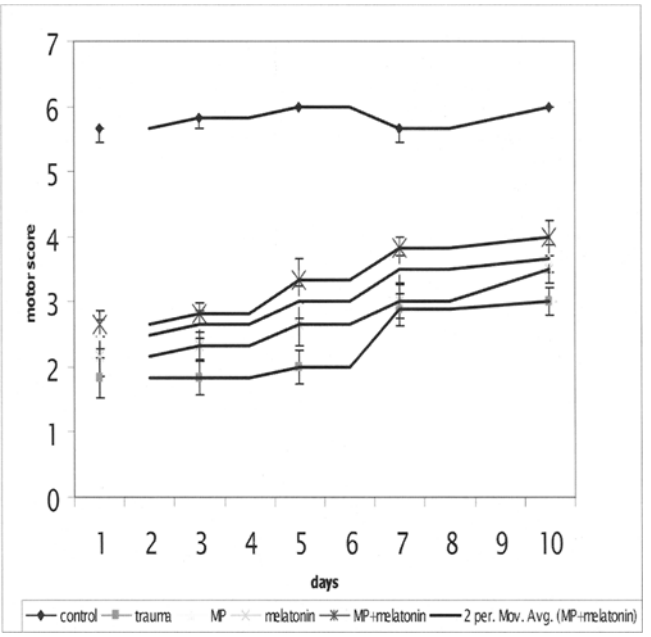

Fig. 2 Motor function score

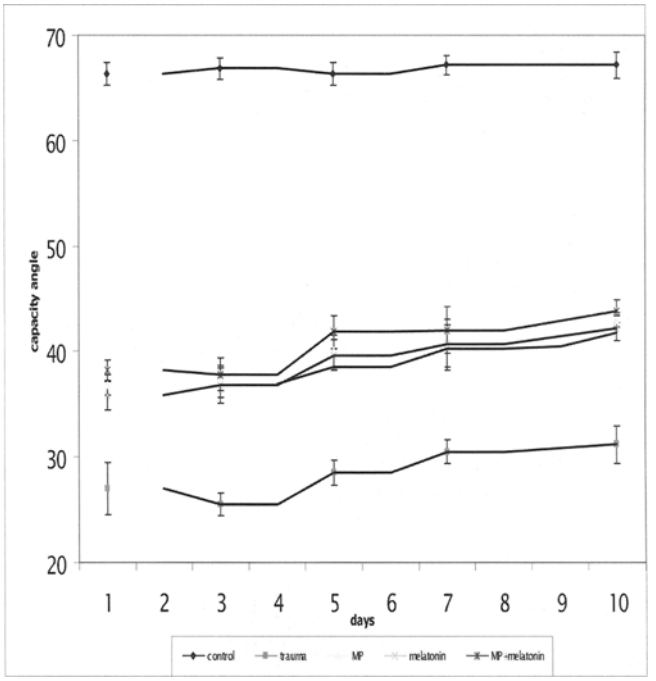

Fig. 3 Inclined angle board score

vation period, but there was no significant difference between the improvement rates (P>0.05). When the differences in the magnitude of change were evaluated, no statistically significant difference was found among them.

Ultrastructural findings

Control group

Ultrastructural findings were normal in the gray and white matter of spinal cord samples obtained in all control rats.

Trauma group

Ultrastructural examination disclosed severe and diffuse interstitial edema in the gray matter. The neurons were apparently edematous and cytoplasmic organelles were not seen. The nucleus usually had no chromatin, and nuclear organelles were unclear. The most typical axonal change observed in the white matter was prominent edema in the axoplasma. The myelin degeneration was severe. Myelin sheath lamellar separation and disruption were frequently seen. Severe degeneration was also observed in the axoplasmic mitochondria.

Four hours postinjury samples

Methylprednisolone-treated group. There were fewer neurons that were severely edematous and the neurons had sparse chromatin. The severely swollen areas were associated with the membrane defects, but cytoplasmic contents were clear when compared with the trauma group. Most of the neurons showed swelling of the mitochondria and disruption of their cristae. The neurofilaments were observed in most of the axons, and even swelling was present but not as severe as the trauma group. Axonal degenerative changes of the white matter were similar to those demonstrated in the gray matter. Myelin sheath lamellar separation and disruption were frequently seen, but myelin clefts were not observed. The swollen mitochondria were abundant, and a few mitochondria had cristae.

Melatonin-treated group. Interstitial edema in the gray matter was more severe than in the MP-treated group. There were fewer, but severely edematous, neurons, but the neurons, nucleus, and cytoplasmic structures were in better condition when compared with the MP-treated group. The axons usually showed mild edema. The axonal cytoplasm was in better condition but degeneration of the myelin sheath was more severe than in the MP-treated group. The mitochondria usually showed slight edema.

Methylprednisolone-melatonin combined treatment group. There was interstitial edema in the gray matter. The appearance of the neurons was similar to the melatonin-treated group. There was light edema in the axons, and the neurofilament density was mainly normal. Vesicular degeneration was seen in the myelin sheath. The mitochondria usually showed slight edema, and cristae could be seen.

Twenty-four hours postinjury samples

Methylprednisolone-treated group. Interstitial edema was observed in the samples obtain at 24 h postinjury. There were several edematous neurons. Despite the existence of edema, all the cytoplasmic organelles were clear. The axoplasmic density was less normal in the white matter but myelin sheath separation was seen. The appearance of neurons and axons was better than in the samples obtained 4 h postinjury. There was light mitochondrial edema, but the appearance of mitochondria was better than the 24-h melatonin-treated group.

Melatonin-treated group. The appearance of the axons and neurons was similar to the samples obtained 24 h postinjury from the MP-treated group. There was light mitochondrial and intracytoplasmic edema.

Methylprednisolone-melatonin combined treatment group. Light edema was observed in the gray matter. The nucleus infrequently had sparse chromatin, and usually clumping was observed. Despite slightly edematous axons in the white matter, there were several axons similar in appearance to those observed in normal spinal cord samples.

Ten days postinjury samples

Methylprednisolone-treated group. The general appearance of the gray matter was closely related to that observed in nontraumatic tissue samples. There was slight interstitial edema, but the mitochondria, axons, and myelin sheaths usually showed normal appearance.

Melatonin-treated group. Cytoplasmic density was normal in the gray matter. Axons were similar to normal appearance. The general appearance was similar to the MP-treated group. In the white matter the axoplasmic density was mainly normal but there was some myelin separation.

Methylprednisolone-melatonin combined treatment group. There was minimal interstitial edema. Chromatin appearance of the nucleus, axoplasmic density, and axonal appearance were similar to the normal samples. There was minimal mitochondrial edema. The global appearance of the spinal cord sample was closely similar to the nontraumatic control group's spinal cord samples.

Lipid peroxidation levels

Figure 4a–c shows the levels of MDA in all groups at the 4th hour, 24th hour, and on the 10th day postinjury. Trauma was found to produce a significant elevation in lipid peroxidation and there was no significant difference between trauma and ethanol groups for all time periods ($P>0.05$ for

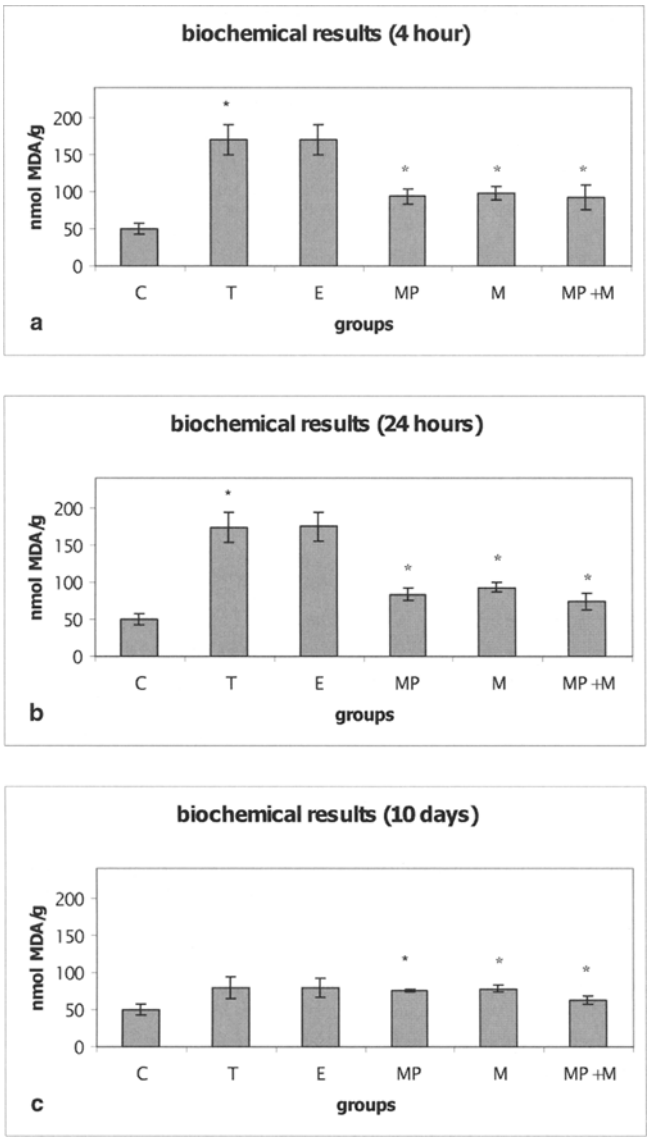

Fig. 4a–c Graphs showing the mean values of malondialdehyde (*MDA*) levels demonstrated at 4 h (**a**; * P=0.004), 24 h (**b**; * P=0.004), and 10 days (**c**; * P=0.006) postinjury. *Vertical lines* indicate standard errors of the mean values. *C* Control group, *T* trauma group, *E* vehicle (ethanol) group, *MP* methylprednisolone group, *M* melatonin group, *MP+M* methylprednisolone+melatonin grou

each). Early treatment with MP, melatonin, and MP+melatonin after trauma significantly decreased the lipid peroxidation compared with trauma groups at 4 and 24 h postinjury (P<0.05 for each). There were no significant differences between the MP-, melatonin-, and MP+melatonin-treated groups at the 4th or 24th hours postinjury (P>0.05 for each). Although there was no significant difference between MP- and melatonin-treated groups on the 10th day postinjury (P>0.05), there was a significant difference between the MP+melatonin-treated group and the other two treatment groups (P<0.05 for each). At the 10th day of injury, the MP+melatonin-treated rats' MDA level was lower than all the groups (P<0.05), while there were no significant differences between the trauma, ethanol, and MP groups and the melatonin-treated groups (P>0.05).

Discussion

The present study showed that an impact of 50 g/cm to the dorsal surface of the spinal cord caused paraplegia and negative electrophysiological responses at the 4th hour after SCI while treatment with MP and melatonin caused some positive responses. Electrophysiological recovery was observed for all groups over a period of 10 days but there was a significant difference between the trauma and treatment groups.

The neuroprotective effects of high-dose MP on SCI have been reported previously. Reduction of lipid peroxidation has been postulated to be the major factor in the improvement of outcome with MP [5]. It suppresses the breakdown of membranes by inhibiting lipid peroxidation and hydrolysis at the site of injury [27]. Recently, melatonin was found to be a highly efficient scavenger of hydroxyl and the peroxyl radicals as well as an electron donor [23]. Melatonin's extreme ability of diffusion is important for its scavenging action since this feature allows it to enter all cells and every subcellular compartment. It has been shown that it may be bound in the nucleus, thereby providing on-site protection to DNA and its high lipophilicity provides oxidative protection in every subcellular compartment [24]. The neuroprotective effect of melatonin has been previously shown in experimental models of SCI by its reducing effect on the content of thiobarbituric acid-reactive substances and myeloperoxidase activity [12, 18]. In addition, Kaptanoğlu et al. have provided ultrastructural information about the neuroprotective effect of melatonin and found that melatonin protects neurons, myelin, axons, and subcellular organelles such as nucleus and mitochondrion [18].

The damaged lesion in the spinal cord develops within 24 h. Neurological conditions get worse for 24 h before the improvement curve begins. It is known that lipid peroxidation products increase soon after injury. In the present study, tissue lipid peroxidation was evaluated by measuring the thiobarbituric acid-reactive substances. MDA, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of lipid peroxidation. MDA is also a well-known secondary product of lipid peroxidation in spinal myelin, glial, and neural membranes. We demonstrated that MDA levels in the MP-treated, melatonin-treated, and MP+melatonin-treated groups were significantly lower than the trauma and ethanol-treated groups. MDA levels reached peak values at the 4th and 24th hours in all the groups, however, they were significantly lower in the treatment groups than the trauma and ethanol groups. On the 10th

day, the MDA levels of all groups were similar except for the MP+melatonin combined treatment group, which had significantly lower MDA levels.

Much experimental and clinical research has focused on these secondary injury mechanisms in an effort to improve neurological outcome following SCI. A reliable test protocol suitable for the injury model used is essential to evaluate functional recovery after SCI [13, 25]. In our experimental design, weight-drop injury caused reversible paraplegia. We observed improvement of motor functions in all groups at the first day postinjury. A significant gradual recovery rate was observed in all the injured rats treated with MP, melatonin, or combined treatment for motor function score and inclined angle board capacity. Although recovery has been detected in both test scores for the trauma group, recovery of the trauma group by itself in inclined board values was not significant. Each treatment group revealed a significant difference of recovery between the first and tenth days, but without any significant difference for the amount of recovery between the groups. At the end of 10 days of observation it can be stated that the effects of MP and melatonin on neurological recovery are the same and the combined treatment of both drugs does not contribute to an increased rate of recovery. The same neurological recovery rate in the rats between melatonin-treated and MP-treated groups and the combined treatment group may be explained by the lack of synergism or additive effect of melatonin and MP in the acute phase of injury.

Our ultrastructural findings support that melatonin, as well as MP, protects neurons, myelin, axons, and cytoplasmic organelles such as nucleus and mitochondrion. The evaluation according to the grading system showed the neuroprotective effects of melatonin, MP, and combined treatment. When melatonin was compared with MP, at posttraumatic 4th hour, melatonin had a more remarkable protective effect on mitochondria while MP was more effective on axon and myelin edema. There is no significant difference between MP and melatonin for the nucleus. The combined treatment results supported the findings. When the melatonin group was compared with the trauma group, at the 4th hour postinjury, melatonin was observed to be effective only on the nucleus and mitochondria. Differences existed between the MP group and the trauma group in all parameters. While melatonin was the most effective agent on mitochondria at the 4th hour, MP was more effective on mitochondria at the 24th hour. Kaptanoğlu et al. have claimed that melatonin has a more significant neuroprotection compared to MP [18]. Since the 48th hour results were better than the 1st hour results, they claimed that the effect of melatonin emerged in the latency period and it could be more effective in chronological recovery. Although our findings support this hypothesis, we showed that melatonin provides an equal neuroprotection to MP at the end of the 10th day. We found that combined treatment with both drugs could not give significantly better results than the single use. Kaptanoğlu et al. have used a single dose (100 mg/kg) of melatonin in their study where they have hypothesized that the ultrastructural findings for melatonin are better than for MP in terms of some parameters. In our study, we used a single 10-mg/kg melatonin dose and ultrastructural findings showed significantly different effects of melatonin and MP only on mitochondria at the 4th and 24th hours of trauma. Nevertheless, neuroprotection effects were similar at the 10th day postinjury for all treatment modalities, suggesting that the melatonin and MP combination may not result in an extra protection of cellular organelles in chronological recovery. Further studies are needed with different melatonin doses to eliminate the discrepancies between the findings since different doses have been a limitation of each study.

Clinical and experimental studies have supported the use of neurophysiological tests as an objective assessment of spinal cord integrity after injury. The cortical somatosensory evoked potential (CSEP) test is a parameter used in the evaluation of the physiological integrity of the IA group fibers, transmitting also the vibration and proprioceptive senses along the posterior tract of the spinal cord. However, in both clinical and experimental studies, it has been observed that CSEP did not correctly predict recovery of motor function after injury [19]. The motor evoked potential (MEP) test is a better measure of postinjury motor function than the CSEP test because it is derived from direct activation of the motor cortex and its efferent pathways [11, 20]. Fehling et al. suggested the MEP test as a useful neurophysiological technique for monitoring the motor tracts of the cord and it reflected the severity of SCI in a dose-response relationship [11]. On the other hand, the CSEP test evaluates mainly afferent pathways to somatosensory cortex. In the present study, both MEP and CSEP have been used in order to evaluate both afferent and efferent spinal tracts which could show the severity of SCI and the effectiveness of pharmacological agents on axonal integrity and recovery of the physiological functions.

The weight-drop trauma model caused severe and standard trauma because no evoked potential response was observed in the rats of the trauma and ethanol groups at the 4th hour postinjury. A gradual increase in amplitudes and a decrease in latency periods were observed for all groups during the study period. However, recorded values of MEP and CSEP showed that electrophysiological recovery was minimal in the trauma and ethanol groups when compared to the treatment groups.

The antioxidative effects of MP and melatonin seem to be at the same level although with different mechanisms. However, at the end of the study the significantly lower levels of MDA in the MP+melatonin combined treatment group in comparison with the other treatment groups may be the result of additive or synergistic effects of different antioxidative mechanisms. This effect might be insignifi-

cant for preventing the pathological process of the injury since maximum tissue destruction takes place immediately and continues for 24 h after the injury [13, 17]. Neurobehavioral, ultrastructural, and electrophysiological results of our study also support this opinion. There was no significant difference between all treatment groups at the 10th day of the study for all parameters.

In the present study, our results confirm that melatonin could be as effective as MP for the prevention of lipid peroxidation after SCI. Even though MP and melatonin have different antioxidative mechanisms, both agents decrease MDA levels to the same level within the first day of the injury. However, at the end of the study, combined treatment with MP and melatonin decreased MDA levels significantly more than the MP- and melatonin-treated groups. This better biochemical recovery may be the result of cumulative effects of the different antioxidative mechanisms of MP and melatonin at the subacute phase of the injury. Although the neurobehavioral, ultrastructural, and electrophysiological recovery was at the same level at the 10th day for all treatment groups, longer follow-up and different dose combinations seem necessary to reach an exact conclusion on the combined treatment for neurological recovery. Nevertheless, even in low doses, melatonin seems to be as effective as MP on secondary damage in spinal cord trauma.

In conclusion, MP, melatonin, and a combination of both agents improved neurological recovery at the same level. Combined treatment resulted in a cumulative effect on lipid peroxidation level in the subacute phase of injury, but did not cause enhanced neurological recovery. Generally, neurological conditions get worse in the acute phase of injury before the improvement curve begins. The limited prevention of the secondary damage in the acute phase of injury can be claimed to be responsible for this result. The dose-response relationship studies for melatonin alone and combined treatment modalities could promise better results.

References

1. Allen AR (1911) Surgery of experimental lesion of spinal cord equivalent to crush injury of fracture dislocation of spinal column. A preliminary report. JAMA 57:878–880
2. Anderson DK, Hall ED (1993) Pathophysiology of spinal cord trauma. Ann Emerg Med 22:987–992
3. Baffour R, Achanta K, Kaufman J, Berman J, Garb JL, Rhee S, Freedmann P (1995) Synergistic effect of basic fibroblast growth factor and methylprednisolone on neurological function after experimental spinal cord injury. J Neurosurg 83:105–110
4. Bracken MB, Shepard MJ, Collins WF, Holford TR, Young W, Baskin DS, Eisenberg HM, Flamm E, Leo-Summers L, Maroon J, et al (1990) A randomized, controlled trial of methylprednisolone or naloxone in the treatment of acute spinal-cord injury. Result of the Second National Acute Spinal Cord Injury Study. N Engl J Med 322:1405–1411
5. Braughler JM, Hall ED (1982) Correlation of methylprednisolone levels in cat spinal cord with its effects on (Na+K)-ATP-ase, lipid peroxidation, and alpha motor neuron function. J Neurosurg 56:838–844
6. Braughler JM, Hall ED (1984) Effects of multi-dose methylprednisolone sodium succinate administration on injured cat spinal cord neurofilament degradation and energy metabolism. J Neurosurg 61:290–295
7. Collins WF (1983) A review and update of experimental and clinical studies of spinal cord injury. Paraplegia 21: 204–219
8. Constantini S, Young W (1994) The effects of methylprednisolone and the ganglioside GM1 on acute spinal cord injury in rats. J Neurosurg 80:97–111
9. Ducker TB, Salcman M, Perot PL Jr, Ballantine D (1978) Experimental spinal cord trauma. I. Correlation of blood flow, tissue oxygen and neurologic status in the dog. Surg Neurol 10:60–63
10. Farooque M, Isaksson J, Jackson D, Olsson Y (1999) Clomethiazole improves hind limb function and reduces neuronal damage after severe spinal cord injury in rat. Acta Neuropathol 98:22–30
11. Fehling MG, Tator CH, Linden RD, Piper IR (1987) Motor evoked potentials recorded from normal and spinal cord injured rats. Neurosurgery 20: 125–130
12. Fujimoto T, Nakamura T, Ikeda T, Takagi K (2000) Potent protective effects of melatonin on experimental spinal cord injury. Spine 25:769–775
13. Gale K, Kerasidis H, Wrathall JR (1985) Spinal cord contusions in the rat: behavioral analysis of functional neurologic impairment. Exp Neurol 88:123–134
14. Hall ED (1993) Lipid peroxidants in acute central nervous system injury. Ann Emerg Med 22:1022–1027
15. Hall ED, Wolf DL, Braughler JM (1984) Effects of a single large dose of methylprednisolone sodium succinate on experimental posttraumatic spinal cord ischemia: dose-response and time-action analysis. J Neurosurg 61:124–130
16. Halliwell B, Auroma OI (1991) DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 281:9–19
17. Kaptanoğlu E, Caner HH, Surucu SH, Akbıyık F (1999) Effect of mexiletine on lipid peroxidation and early ultrastructural findings in experimental spinal cord injury. J Neurosurg 91:200–204
18. Kaptanoğlu E, Tuncel M, Palaoğlu S, Konan A, Demirpençe E, Kılınç K (2000) Comparison of the effects of melatonin and methylprednisolone in experimental spinal cord injury. J Neurosurg (1 suppl) 93:77–84
19. Levy W, McCaffrey M, York D (1986) Motor evoked potentials in cats with acute spinal cord injury. Neurosurgery 19:9–19
20. Macdonell RA, Donnon GA, Bladin PF (1989) A comparison of somatosensory evoked potentials and motor evoked potentials. Am Neurol 25:68–73
21. Menendez-Pelaez A, Poeggeler B, Reiter RJ, Barlow-Walden L, Pablos MI, Tan DX (1993) Nuclear localization of melatonin in different mammalian tissues: immunocytochemical and radioimmunoassay evidence. J Cell Biochem 53:373–382

22. Mihara S, Uchiyama M (1978) Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 86:271–278
23. Pieri C, Marra M (1994) Melatonin; a peroxyl radical scavenger more effective than vitamin E. Life Sci 55: 271–276
24. Reiter RJ (1996) Functional diversity of the pineal hormone melatonin: its role as an antioxidant. Exp Clin Endocrinol Diabetes 104:10–16
25. Rivlin AS, Tator CH (1977) Objective clinical assessment of motor function after experimental spinal cord injury in the rat. J Neurosurg 47:577–581
26. Tator CH, Fehlings MG (1991) Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 75: 15–26
27. Young W, Flamm ES (1982) Effects of high-dose corticosteroid therapy on blood flow, evoked potentials and extracellular calcium in experimental spinal cord injury. J Neurosurg 57:667–673