Comparative evaluation of the local application of dimethyl sulfoxide in an experimental spinal cord injury model

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Abstract

Aim: To investigate the efficacy of the local application of dimethyl sulfoxide (DMSO) in an experimental spinal cord injury model.

Material and Methods: This study included 20 Sprague–Dawley rats (12 males and 8 females) that were aged 4–6 months and weighed 160–300 g. The control and experimental groups had equal number of rats (n = 10). Ketamine hydrochloride (50 mg/kg) and diazepam (2 mg/kg) in saline solution were intraperitoneally administered. A rod bearing, glass tube through which the weight was passed, and modified Allen trauma device were used to induce trauma. The rats then developed post-traumatic flask paraplegia. DMSO was topically applied to the epidural space after a two-level laminectomy. The global motor performance of the rats was evaluated.

Results: The motor performance of the experimental and control groups was assessed after trauma. Results showed that the rats presented with significantly decreased motor performance. In particular, the performance of the experimental group improved over time, and recovery accelerated after the fifth day. Based on a histological examination of the lesion sites after the tenth day, the experimental and control groups significantly differed in terms of global motor performance.

Conclusion: The efficacy of the agent was evaluated via global motor performance assessment and histological examination. In the experimental group, a statistically significant difference was observed in terms of global motor performance, which was assessed using the tilted floor method, on the fifth day after trauma. Histologically, no statistically significant difference was noted between the two groups.

Keywords: Dimethyl sulfoxide; laminectomy; spinal cord injury

INTRODUCTION

Spinal cord injury (SCI) is a significant neurological problem worldwide, with an incidence of 14–40/1,000,000 individuals (1). It causes neurological complications, such as motor and sensory loss, bladder and intestinal dysfunction, spasticity, neuropathic pain, and autonomic dysreflexia, and systemic problems, including deep vein thrombosis and osteoporosis (2-5). These complications lower quality of life and functional capacity. Although the number of individuals with SCI increases annually, the treatment guidelines do not recommend any medications considered effective for such condition (6-8). In fact, only stabilization and decompression surgeries are indicated (9).

Acute SCI has two phases: primary mechanical injury and secondary cellular injury (8). In primary mechanical injury, spinal cord compression, which is characterized by sudden and irreversible mechanical damage, occurs. After the primary injury, secondary complications, such as bleeding, vasospasm, ischemia, necrosis, edema, excitotoxicity, inflammation, lipid peroxidation and free radical production, and apoptosis, develop. Moreover, they have a relatively slow course and are mainly observed at the cellular level (10). These mechanisms increase the risk of developing neurological problems among individuals with pathologic conditions, such as demyelination, glial scar, and apoptosis (10). Although one might avoid primary damage with the use of preventive methods alone, actual treatments aim to reduce or prevent secondary damage. Based on the mortality and morbidity rates of patients with SCI worldwide, we found that there is no effective method for preventing or decreasing the occurrence of...
secondary damage. Thus, new and effective treatment strategies should be developed.

Dimethyl sulfoxide (DMSO) is an agent used as a solvent to dissolve various medications, and various pharmacologic studies have shown the biological effects of this agent (11). The biological activities of DMSO have neuroprotective effects (12) against hydroxyl radical scavenging, inflammation, edema, platelet aggregation and adhesion, and glutamate-induced neuronal cell death (12).

DMSO studies conducted within the last 30 years generally focused on traumatic brain damage and stroke (11). Previous studies worldwide are either old dated or written in the Russian language. Thus, data about DMSO are limited. In relation to this, the actual number of studies about the effect of DMSO on SCI is limited. Hence, the role and mechanism underlying the effect of DMSO in the secondary damage of SCI could not be fully understood.

The current study aimed to determine whether the topical application of DMSO has direct and/or indirect biological effects on the functional and histological outcomes of secondary damage of SCI.

MATERIALS AND METHODS

Animals

This study was approved by the ethics committee of the institution where the work was performed (decision no: 2015/49, date: 30.07.2015). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed, and the procedures performed were in accordance with the ethical standards of the institution or laboratory at which the studies were conducted.

In total, 20 Sprague–Dawley rats (12 males and 8 females) that were aged 4–6 months and weighed 160–300 g were used in this study. The rats were randomly allocated into two groups (n=10 each). The two groups were homogeneous in terms of biological and physiological characteristics.

Trauma Device

The rats were positioned with all four feet on a steel ring at a height of 10 cm from a flat floor. A steel bar, which was 50-cm long and mounted at 90° to the steel ring, was used to adjust the height of the ring in increments of 2.5 cm. The setup comprised a rod bearing, glass tube through which weight was passed, and modified Allen trauma device (consisting of a 20-cm-long glass tube weighing 2.5 g) made of lead (impact surface, 3 mm in diameter) (Figure 1).

Pharmacological Agent

DMSO (Merck KGaA, 64271 Darmstad, Germany) was applied topically to the epidural space during laminectomy.

Microscopic Imaging and Analysis

The Carl Zeiss OPMI-IH operation microscope was used for analysis.

Figure 1. The arrows indicate the trauma sites in the control and experimental groups

Intervention

General anesthesia was induced via the intraperitoneal administration of ketamine hydrochloride (50 mg/kg) (Ketalar, Parke Davis Eczacibasi, Turkey) and diazepam (2 mg/kg) (Diazem, 2-mL ampule, Deva Holding A.S., Turkey) in saline solution. The rats were placed in prone position on a plastic plate (40 x 40 cm), and the four extremities of the rats were fixed to the plate. All surgical procedures were performed under an operation microscope using aseptic technique.

A 3 x 3-cm area in the mid-thoracic region was shaved and disinfected with 10% povidone iodine. A 3-cm incision was made through the skin, subcutaneous layer, and fascia. The paraspinal arches were stripped via blunt dissection on both sides, and the laminae were exposed. A two-level laminectomy was performed microsurgically (Figure 2). The trauma device—a cylindrical-shaped lead weighing 2.5 g with a 3-mm diameter impact surface—was lowered over the spinal cord from a height of 20 cm via a glass tube through which it could easily pass. Thus, post-traumatic flask paraplegia was induced.

Figure 2. Image of the intraoperative view of the two-level laminectomy
Administration of the Pharmacological Agent

Approximately 15 min after surgery, 2 g/kg (about 1 cc) of 40% (v/v) DMSO in saline solution was administered to each epidural space at the trauma site.

Postoperative Care

The rats were transferred to a cage containing fine wood shavings maintained at 24°C. All rats received 3 mg/kg of gentamicin for 3 days, and prophylactic therapy was provided to prevent urinary tract infection. During the experimental period, all rats were fed ad libitum with pellets containing 21% crude protein (Purina) and had access to regular drinking water.

Evaluation of Motor Function

Global motor performance was evaluated using the tilted area method. The assessment was performed on a wooden floor that could reach a maximum of 90° and could be gripped by the rats. Then, the rats were placed on this floor with their heads oriented upward, and the inclination was increased in 5° increments to ensure that the rats could hold on for 5 s. If they could not hold on, the inclination was reduced by 2.5°. The measurements were performed daily.

Collection of Samples

After the motor function test, the rats were sacrificed via the intracardiac injection of high-dose sodium pentothal 10 days after surgery. A five-level laminectomy focusing on the trauma site was performed, and the cord and dura, including the region 1 cm proximal and 1 cm distal to the lesion, were removed. The specimen was fixed in 10% formalin for evaluation under the light microscope. The medulla spinalis specimens were then embedded in paraffin and blocked.

Histological Analysis (Preparation of Specimens)

The rats were sacrificed after the evaluation of global motor performance on the tenth day after trauma. The trauma region was centralized to make the lesion in the middle thoracic region wider than the location, and the spinal cord and dura 1 cm distal to the traumatic site were removed and fixed in 10% formalin solution.

A macroscopic evaluation of the pathological material was performed in a neuropathology laboratory. The dura was opened to reveal the covered arachnoid. The prepared paraffin sections were stained with hematoxylin and eosin and Masson’s trichrome dye for microscopic analysis.

Statistical Analysis

All data were analyzed using the Statistical Package for the Social Sciences software for Windows version 20.0 (IBM Corp., Armonk, NY, USA). The Mann–Whitney U (z) test was used to compare descriptive data (mean, standard deviation, minimum, and maximum) with a non-normal distribution via a quantitative analysis. A p value < 0.05 was considered statistically significant.

RESULTS

Prior to inducing trauma, the assessment of motor performance revealed that the experimental and control groups could grip on a 81.50° and 82.50° slope, respectively. The mean post-traumatic measurements in the control group were 29° 3 h after trauma and 38.25°, 41.75°, and 47.75° on the first, fifth, and tenth day after trauma, respectively (Figure 3). Meanwhile, the post-traumatic measurements in the experimental group were 29.50° 3 h after trauma and 41.75°, 47.50°, and 54° on the first, fifth, and tenth day after trauma, respectively. The decrease in motor performance did not significantly differ between the control and experimental groups prior to trauma and 3 h or 1 day after trauma. However, the motor performance of both groups improved over time, which was evident in the experimental group. The recovery of the rats accelerated after the fifth day. On the fifth and tenth day after trauma, the experimental group had a significantly higher performance level than the control group (Figure 3 and Table 1).

Figure 3. Graphical comparison of the motor performance of the control and experimental groups

Histological Results

The macroscopic evaluation revealed that the length and diameter of the spinal cord were 2.5 and 0.2–0.3 cm, respectively. A thin layer of dark brown-red blood was observed in the subarachnoid region.

Moreover, numerous phagocytic monocytes were noted in the trauma site. The phagocytic cells had large, acidophilic, vacuolar cytoplasms and marginally located cores. Thus, parenchymal necrosis in this region was considered. Partial petechial hemorrhages were observed in the white matter, particularly in the dorsal area, in the regions proximal and distal to the lesion (Figures 4 and 5).

The histopathological evaluations of the lesion sites on the tenth day revealed that the global motor performance did not significantly differ between the experimental and control groups. In addition, epidural fibrosis was reduced in the experimental group compared with the control group.

Adverse Events

The rats were alive for 10 days. However, no significant adverse event was observed in both groups during the study period.
**DISCUSSION**

In the current study, the beneficial effects of DMSO on motor performance were observed particularly on the fifth day. Moreover, the experimental group experienced a significant decrease in epidural fibrosis. Based on this finding, future studies of patients with SCI lesions can use DMSO and the combination of DMSO and other agents as they might be a good therapeutic option. Moreover, there are no studies written in the English language that assessed the effects of the topical application of DMSO and that included a control group. Thus, to the best of our knowledge, this study first recruited a control group and evaluated the effects of topical DMSO.

Although no study has assessed the local application of DMSO in humans with SCI, it has been used in experimental SCI models (11,13). In the experiments about SCI-evoked animals, DMSO was applied intravenously after 2 h, and results showed that it might prevent paralysis (14,15).

Studies about animals with experimentally evoked SCI have shown that DMSO has biological effects (16). Moreover, the findings of studies that compared the effects of agents, such as steroids, hyperbaric oxygen, mannitol, and urea, were in favor of DMSO (12). DMSO was found to have significant advantages as it increased sensory-motor improvement, facilitated early regain of somatosensory evoked potentials, decreased neural damage after trauma, reduced tissue swelling after trauma, and improved muscle strength (13,17).

In other studies, DMSO protected the axons after trauma, decreased inflammation in myelin sheath and tissue cavitation, and increased blood flow to the spinal cord (16,17).

In animal studies, medications preventing the Na+ influx to the neurons have strong neuroprotective effects. Several medications that have these characteristics were found to prevent cerebral ischemia "For example, mexiletine is one of these drugs. The protective effect of mexiletin in cerebral ischemic injury is associated with Na + channel blockade, Ca2+ channel blockade and antioxidant effect (18). DMSO is an Na+ canal blocker, and this result can partly explain the neuroprotective effects of DMSO against...
physical trauma and ischemic stroke (1,19). Moreover, DMSO might be useful in cases of increasing intracranial pressure, such as head trauma and hemorrhagic stroke cases (12,20,21).

The excitotoxic process evoked by glutamate might destroy or damage the neurons (22). At clinical doses, DMSO prevents excessive Ca2+ inflow into the cells and N-methyl-D-aspartate and α-amino-3-hydroxy-5-methylisoxazole-4-propionate activated with glutamate during oxidative or metabolic stress (22).

Another study has shown that DMSO has synergic effects on fructose 1,6-diphosphate. Moreover, it can prevent the production of oxygen and free radicals (23) and progressive brain edema caused by injury by restoring ATP levels, which is decreased in ischemia (20,23).

In an experimental study of cerebral ischemia, DMSO was found to be a strong platelet de-aggregator, and when this agent is combined with vasodilator prostacyclin, it increased the cerebral blood flow in the experimental group compared with the control group (24).

Safety is the most important factor that must be considered in medical therapy. DMSO has mild adverse effects, which vary based on the concentration and method of application (11). Although the toxic reactions of DMSO are rarely observed, dose-related hemolysis and hemoglobinuria developed after the intravascular application of the agent. However, renal functions were not affected (14). In a study about DMSO-cryopreserved peripheral blood stem cell infusion, the agent was found to be safe even when used in patients who had a previous history of cerebral disease (21). The garlic-like smell of DMSO may limit its use in experimental animal studies and in humans if tested.

**CONCLUSION**

There is no available treatment option for SCI other than stabilization and decompression surgeries. In the current study, no significant difference was observed based on the histopathological comparison of trauma regions in the experimental and control groups on the tenth day after trauma. However, the significant difference in global motor performance indicates that similar studies must be carried out. The experimental group had significantly better motor performance than the control group. These results are considered acceptable if they were observed in human participants. However, DMSO studies, particularly those about SCI, were old dated. Thus, the current study might shed light to actual medical studies about SCI and those that conducted a re-analysis of the use of DMSO in more contemporary trauma models, imaging methods, and pharmacologic, functional, and histological examinations. Other than decompression and stabilization after spinal cord damges, medical treatment opportunities are discursive for this reason, more experimental drugs should be worked.

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**REFERENCES**


