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Failure of Topical DMSO to Improve Blood Flow or Evoked Potentials in Rat Spinal Cord Injury

Dimethyl sulfoxide (DMSO) is a well-known hydroxyl radical scavenger, which is readily absorbed through biological membranes. We studied the effects of locally applied DMSO on acute spinal cord injury. Either 10% DMSO in saline (n=8) or saline alone (n=7) was applied directly to the exposed cervical spinal cord of rats 1 hour after clip compression injury of 26 g force for 1 minute. The outcomes measured were spinal cord blood flow and evoked potentials. Spinal cord blood flow was not significantly different between these two groups. Although the evoked potentials showed spontaneous recovery after injury, there was no significant difference between the groups. In this study we failed to show any beneficial effects from topical application of high-dose DMSO on spinal cord blood flow or evoked potentials after acute spinal cord injury.

Key Words: *Dimethyl sulfoxide; Evoked potentials; Regional blood flow; Spinal cord injuries; Administration, topical*

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INTRODUCTION

It has been postulated that secondary injury mechanisms may progressively worsen the initial primary injury, and that the interruption of these secondary mechanisms is a potentially fruitful goal of modern pharmacotherapy for acute spinal cord injury (SCI). Since vascular damage and free radical generation are considered to be important secondary injury mechanisms in the pathophysiology of SCI (1-6), it was logical to examine the usefulness of the free radical scavenger, dimethyl sulfoxide (DMSO), which has been reported to be effective in head injury (7, 8) and cerebral ischemia (9-11). Its ability to scavenge free radicals (12-14) and protect cell membranes from physical and chemical injury (13, 15-17) may underlie its effectiveness. It has also been shown to increase cerebral blood flow when administered parenterally (18, 19) and induce vasodilation after topical application of high concentration (20). While some experimental studies on SCI showed a beneficial effect (21-25), others failed (26, 27). Acute systemic toxicity is one of the limiting factors in high-dose experimental trials. Thus, it was considered worthwhile to explore the usefulness of topical application of high-dose DMSO because it penetrates well into the tissue.

MATERIALS AND METHODS

Fifteen male Wistar rats weighing 350-450 g were used in the experiment. Aenesthesia was induced by an intraperitoneal injection of 75 mg/kg alpha-chloralose and 525 mg/kg urethane. The right femoral artery was cannulated with PE50 tubing to monitor blood pressure. Both femoral veins were cannulated with silastic tubing to enable delivery of all fluids and drugs. After tracheotomy was performed, the rats were given a neuromuscular blocking agent (pancuronium bromide) through intravenous injection, and mechanically ventilated with a mixture of N_2O:O_2 (2:1). An initial dose of 0.7 mg pancuronium bromide was given followed by 0.1 mg/100 g dose every 30 minutes. A continuous infusion of fluids was maintained through the femoral vein at a rate of 7.1 mL/kg/hr, consisting of 93% saline, 5% albumin and 2% sodium bicarbonate solution (7.5% USP). Three hours after the initial intraperitoneal administration of alpha-chloralose with urethane, the above mixture of infusion fluids was substituted with one containing a maintenance dose of alpha-chloralose with urethane. The drug concentration was such that it provided 1/6 of the initial dose every hr through continuous infusion. Mean arterial blood pressure, heart rate, rectal temperature, hemato-

crit, and arterial blood gases were also monitored.

For evoked potential measurements, a right parietal burr hole was made at 1 mm posterior and 2 mm lateral to the bregma for the somatosensory evoked potentials (SSEPs). A platinum ball electrode, 0.8 mm diameter, was inserted through the burr hole into the epidural space overlying the sensorimotor cortex and positioned 1 mm posterior and 3 mm right of the bregma. An occipital burr hole was made; 1 mm inferior to the external occipital protuberance and 2 mm left to the midline for the cerebellar evoked potentials (CEPs). A platinum ball electrode was then inserted through this hole and placed 3 mm left of the midline. The sciatic nerve was exposed for peripheral nerve stimulation.

For recording SSEPs and CEPs, the left sciatic nerve was stimulated with a bipolar electrode using a cathodal stimulation of 5 mA, 4.05 Hz and duration of 0.05 msec. With a platinum ball electrode (0.8 mm diameter) SSEPs were recorded from the right sensory cortex and CEPs were recorded from the left paramedian lobule of the cerebellar hemisphere. An Ag-AgCl disc electrode placed between the hard palate and the tongue acted as a reference. A needle electrode was inserted subcutaneously between the stimulating and recording electrodes and was connected to a floating ground. A total of 200 SSEP and CEP responses were recorded at a bandwidth of 30 to 3,000 Hz, averaged, and replicated.

A laminectomy at C7-T1 was made for the purpose of measuring spinal cord blood flow (SCBF). SCBF was measured by the hydrogen clearance technique. The dura at C7-T1 was opened and two platinum/iridium microelectrodes with a 10 μm tip diameter were inserted into the dorsal column of the spinal cord at the T1 segment, 0.5 mm lateral to the dorsal vein to a depth of 500 μm using a micromanipulator. An Ag-Agcl disc electrode was placed subcutaneously as a reference. The administration of 8% hydrogen gas for 15 minutes allowed for saturation of the animal. The hydrogen gas was then removed and the initial slope index method was used to measure the SCBF.

After baseline SCBF and EP recordings were made, the animals underwent a 1 minute 26 g clip compression injury at the T1 level using our modified aneurysm clip. After measuring of SCBF and EPs, the rats were randomly assigned to DMSO or saline groups. At 1 hr after injury either 10% DMSO in saline (n=8) or saline alone (n=7) was topically applied to the exposed spinal cord for 3 hours and removed at a rate of 0.5 mL/min with a peristaltic pump. The volume of the spinal cord bath was 1 mL, so that a complete change of solution took 2 minutes. Epidural temperature was maintained in the range of 34-35℃.

RESULTS

Physiological parameters

Table 1 presents the mean changes in physiological parameters during the experiment. pH, $PaCO_2$, and PaO_2 showed no significant change during the experiment. Mean arterial blood pressure briefly increased after the injury, and then significantly decreased (to 65-70% of baseline) in all animals and remained low until the end of experiment. The hematocrit decreased serially during the experiment in both groups. There were no

Table 1. Changes in physiological parameters during the experiment

Time group	MABP (mmHg)	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Hematocrit (%)	Temperature (℃)
Baseline						
Control	115.4±4.1	7.39±0.01	41.5±1.6	211±27	39.2±1.7	37.2±0.2
DMSO	116.7±4.2	7.37±0.01	40.0±0.9	208±27	42.3±0.7	37.0±0.3
Postinjury 1 hr						
Control	78.4±2.6*	7.40±0.01	38.0±0.7	194±14	32.0±1.2*	36.9±0.1
DMSO	78.3±5.3*	7.40±0.01	37.0±1.4	213±14	35.7±1.1*	37.2±0.2
Postinjury 2 hr						
Control	80.2±3.1*	7.41±0.01	37.6±1.5	213±21	27.8±1.7*	37.4±0.2
DMSO	76.6±5.9*	7.40±0.01	38.1±0.9	207±19	33.7±1.4*	37.3±0.2
Postinjury 3 hr						
Control	88.0±4.9*	7.41±0.01	39.6±1.0	229±23	28.4±1.7*	37.3±0.1
DMSO	76.5±7.0*	7.40±0.01	38.5±1.2	228±17	28.5±1.1*	37.1±0.3
Postinjury 4 hr						
Control	81.0±3.0*	7.40±0.01	38.0±0.7	220±24	27.4±1.7*	37.1±0.2
DMSO	71.5±5.0*	7.41±0.01	41.4±1.6	230±17	28.0±1.1*	37.4±0.2

Values are expressed as mean±SEM.
Asterisk indicates a significant difference compared with baseline value ($p<0.05$).

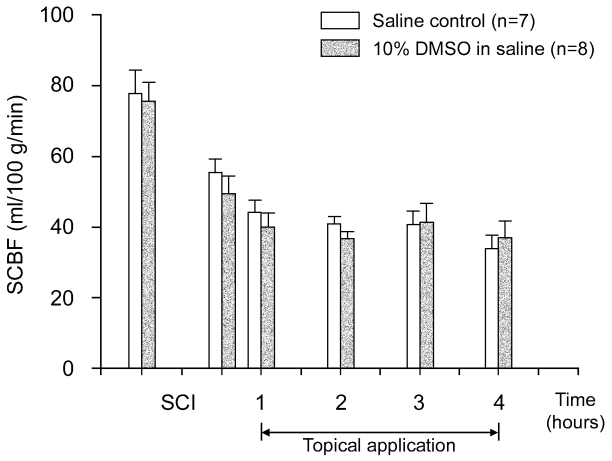

Fig. 1. SCBF (mean±SEM) in each experimental group at pre-injury and during topical application of DMSO or saline. There was no significant difference between the groups.

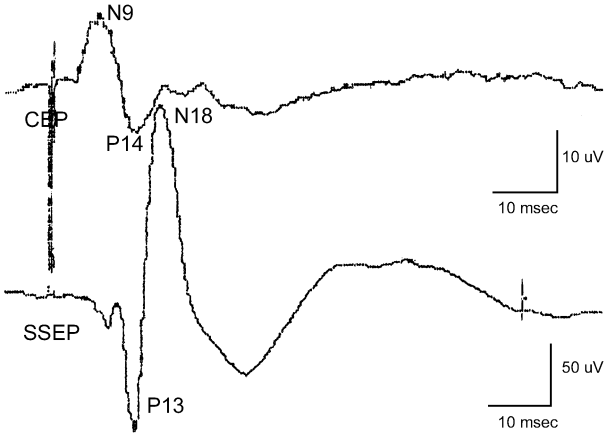

Fig. 2. Normal cerebellar (CEP) and somatosensory evoked potential (SSEP) on stimulation of left sciatic nerve.

specific changes of physiological parameters related to the topical application of DMSO.

SCBF

The preinjury SCBF did not vary significantly between the experimental groups (Fig. 1). The decline in SCBF was significant even during the first 1 hr after injury in both groups (to 65% of baseline). SCBF in the DMSO group did not differ from the control group and remained low without evidence of recovery during the experiment (52-55% of baseline).

Evoked potentials

Normal CEP and SSEP on stimulation of left sciatic nerve is illustrated in Fig. 2. The SSEPs were markedly changed immediately after injury in all animals, disappeared completely in 3 animals (1 in the control group and 2 in the DMSO group), and then partially recovered during the first hr. Then the latency of P13 and N18 increased gradually. When compared with the latency at 1 hr, the latency at 3 and 4 hrs after injury were significantly delayed ($p<0.05$, paired t-test). However, the amplitude did not show a significant change in any group. There was no significant difference in SSEP findings between the two groups at any time interval (Fig. 3).

The amplitude of the CEP decreased markedly after injury in all animals. However, following changes of the amplitude after initial decrease were minimum. There was no significant changes of latency as shown in SSEP. The difference in CEP findings between the two groups at each time interval was not significant (Fig. 4).

DISCUSSION

Since DMSO readily crosses most tissue membranes and enhances the penetration of other molecules, it has often been used as a topical agent or a vehicle for therapeutic agents. Its anti-inflammatory action has been applied in the treatment of interstitial cystitis, granulomatous disease of the skin, arthritis, and even in meningitis (28-30). Reducing ischemia-reperfusion injury of the intestine is another example of the benefical effect of topical use (31). The vasodilating effects of high concentrations of topical DMSO were reported in the pial arterioles of cat cerebral cortex and were mostly due to its hypertonicity (20). It has been successfully applied in pedicle flap surgery (32, 33) and enhanced the flap survival of rat skin flaps subjected to various degrees of ischemia (34). DMSO has been reported to promote the formation of ATP from ADP (35), inhibit Na-K ATPase (36) and oxygen uptake (30).

However, there have been some reports that the systemic use of DMSO confers no protection after cerebral ischemia (37-39). Also in a chronic study DMSO interfered neurite growth in vitro (40), and inhibited functional recovery from cryoinjury of peripheral nerve (41). A severe reversible encephalopathy developed after infusion of DMSO in cancer patients who received peripheral blood stem cells cryopreserved in 10% DMSO (42).

The proper concentration of DMSO in various studies varied with the route of application. When applied topically on the skin, a 40-70% solution was used. Whereas instillation into a hollow viscus such as the intestine, or urinary bladder has been performed with a 1-10% concentration. For intravenous administration, 10-40% has

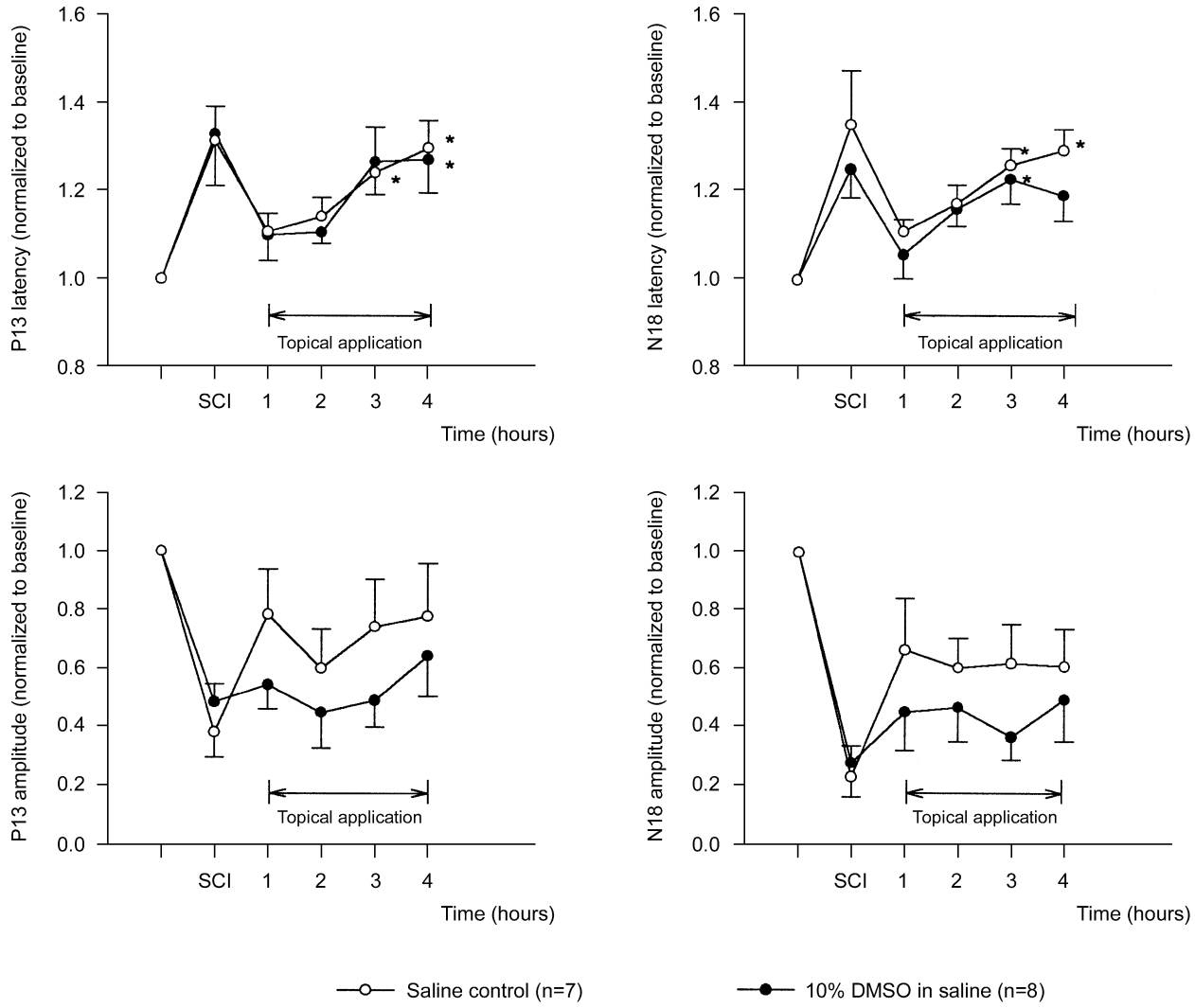

Fig. 3. The latency of P13 and N18 of SSEP recording showed a significant increase after injury followed by some recovery for an hour and then a gradual increase. The values at three and four hours after SCI were significantly higher than at 1 hour post-injury. The amplitude of P13 and N18 decreased immediately after injury and then partially recovered later. In contrast to latency, there was no significant late deterioration. Topical application of 10% DMSO did not show any difference in SSEP from the control. Values are expressed as mean±SEM. Asterisk indicates significant difference ($p<0.05$) compared with 1 hour after injury.

generally been used and has been relatively safe and well tolerated when delivered below 1-2 g/kg bolus. In our experience in the rat, intravenous infusion of 1.5 g/kg given during a 10-15 min period often produced severe arterial hypotension which recovered spontaneously after the infusion was stopped.

However, some effects on the neural tissue has been provoked by high concentration. For example, synaptic facilitation of sympathetic ganglion was achieved by 3-10% concentration (43). Conduction of peripheral nerve C fibers was reversibly blocked by a minimum concentration of 9% (44). Depressing of the increases in membrane permeability induced by acetylcholine, glutamate and GABA was shown by 8.3% (45). Cortical spreading depression evoked in rat cerebral cortex was reversibly suppressed with a 20% concentration (unpublished data). Among various pharmacological uses for extraneural tissue, the best protective action for cryopreservation of cells was obtained by 10% DMSO.

In the present experiment, the topical application of 10% DMSO did not significantly increase SCBF or produce recovery of EPs after SCI at the T1 segment. Although the mean amplitude of P13 and N18 peak of SSEP were reduced in the DMSO group suggesting a possible toxic effect, the difference from the control group was not significant. These findings may be related

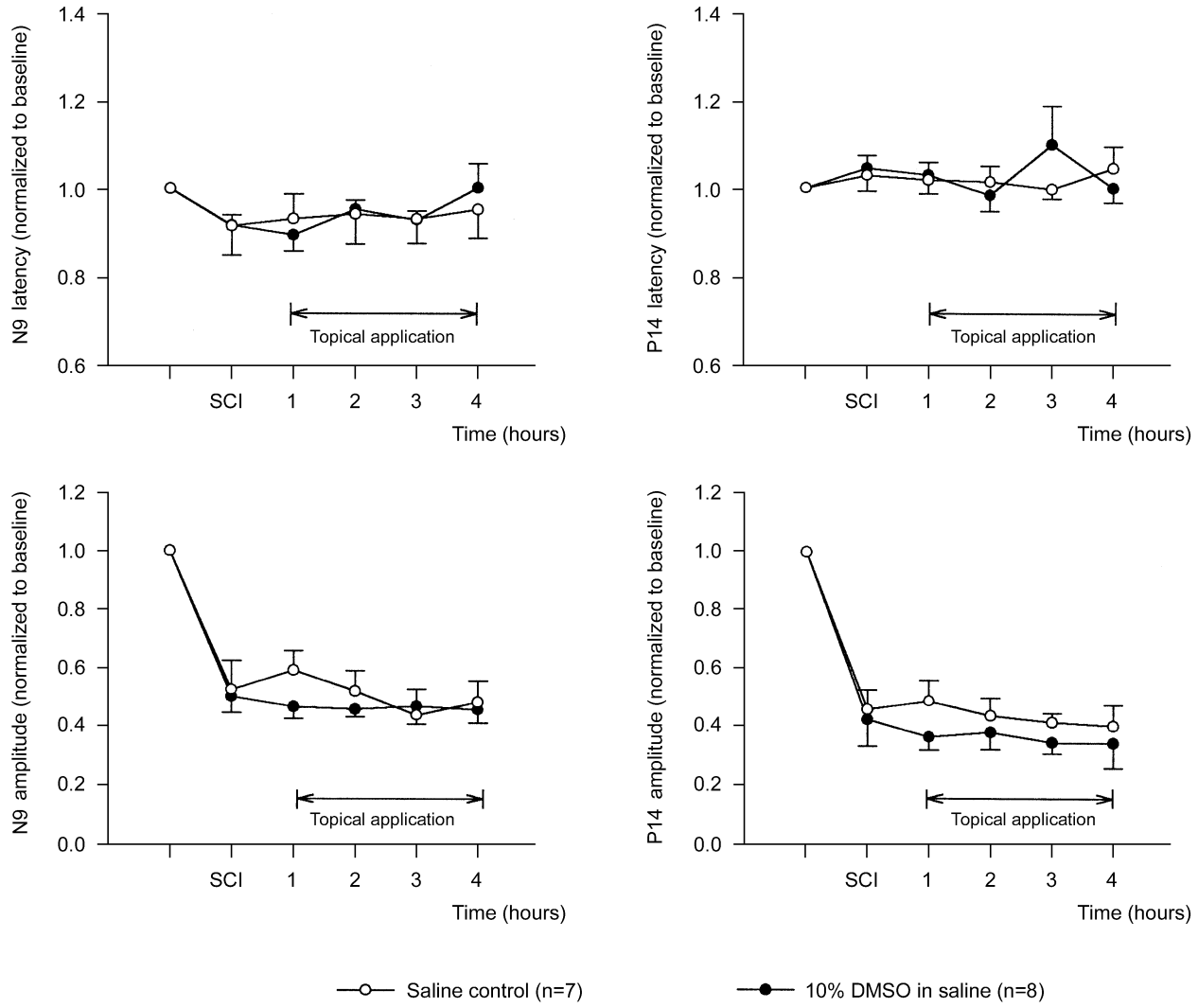

Fig. 4. The latency of N9 and P14 of CEP recording did not show any significant changes after injury. There was no initial delay or late deterioration. However, the amplitude of N9 and P14 decreased markedly immediately after injury, and showed no evidence of recovery. There were no significant differences between the DMSO and saline control group. Values are expressed as mean±SEM.

to the reversible conduction block of nerve fiber achieved in a nerve bath (44). In contrast to the peripheral nerve conduction study in a nerve bath, the tissue concentration of DMSO should have been much lower after topical application to the exposed spinal cord, although continuous delivery probably maintained the concentration.

The lack of beneficial effects, on either the recovery of SCBF or EP with topical use of DMSO, did not totally eliminate any potential benefit after SCI. Systemic hypotension induced by the loss of sympathetic tone after SCI would mask the vasodilatory effect of DMSO. Also the therapeutic window of DMSO for SCI may be very brief and the optimum time for drug delivery may be very soon after SCI. Onset of treatment 1 hr after SCI may have been too late to prevent free radical induced injury.

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