Effects of a single large dose of methylprednisolone sodium succinate on experimental posttraumatic spinal cord ischemia

Dose-response and time-action analysis

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The ability of a single large intravenous dose of methylprednisolone sodium succinate (MPSS: 15, 30, or 60 mg/kg) to modify the evolution of lumbar spinal cord ischemia in cats undergoing a contusion injury of 500 gm-cm is examined. Repeated measurements of spinal cord blood flow (SCBF) in the dorsolateral funiculus were made via the hydrogen clearance technique before and for 4 to 5 hours after injury. The mean preinjury SCBF for all animals was 12.29 ± 0.77 ml/100 gm/min. Following injury, SCBF began to decrease progressively in vehicle-treated animals to a level of 7.71 ml/100 gm/min, a fall of 37.3%. In contrast, cats that received a 30-mg/kg intravenous dose of MPSS at 30 minutes after injury maintained SCBF within normal limits (p < 0.05 at 3 and 4 hours after contusion). A 15-mg/kg MPSS dose was less effective at preventing posttraumatic white matter ischemia, and a 60-mg/kg dose was essentially ineffective.

It was determined that the 30-mg/kg MPSS dose was optimal for supporting SCBF when the drug was given at 30 minutes after spinal trauma, and a second series of experiments was carried out to examine the ability of this dose, when given at longer latencies, to improve decreased flow. Methylprednisolone given at 1½ hours after injury in four cats produced a slight (12.7%) but transient improvement in SCBF, and when administered at 4½ hours in another three animals was totally ineffective. These results show that MPSS in a 30-mg/kg dose can prevent posttraumatic spinal cord ischemia. However, it would appear that the ability of the steroid to reverse the ischemia once it has developed is limited, and probably lost, within a few hours of onset. This further suggests that the ischemic process is irreversible and underscores the need for early treatment with a large MPSS dose in order to prevent full development of ischemia and to promote neurological recovery.

KEY WORDS • spinal cord injury • spinal cord blood flow • methylprednisolone
Methylprednisolone in experimental cord ischemia

accumulation, suggesting a beneficial, but biphasic, effect of this steroid on SCBF as well.8

Based upon these reports, another study was conducted in cats in which the early development of thoracic spinal white matter ischemia (assessed by repetitive hydrogen (H2) clearance measurements) was prevented by a single 30-mg/kg intravenous dose of MPSS given 45 minutes after contusion injury.32 A 15-mg/kg dose was less effective. In addition, the maintenance of SCBF was correlated with a greater incidence of somatosensory evoked potential (SEP) restoration and a lessened posttraumatic calcium accumulation within the injured cord segment. This present investigation, also in cats, repeated the earlier study42 using a wider MPSS dose range. The ability of a single large intravenous dose of MPSS to reverse the white matter ischemia, once it has developed significantly, was also examined.

Materials and Methods

Twenty-five adult mongrel cats of either sex, weighing 2.2 to 3.7 kg each, were anesthetized with sodium pentobarbital (30 mg/kg via the saphenous vein), with supplemental doses given as needed throughout the subsequent experiment. A tracheotomy was performed and a catheter inserted into the right carotid artery for blood pressure monitoring. The left sciatic nerve was exposed by blunt dissection of the popliteal fossa, and a spinal electrode was carried out to expose the L-3 spinal cord segment. The animal was then placed in the prone position in a Kopf 1780 spinal unit* and rigidly fixed in place by hip spikes, a clamp on the L-2 spinous process, and a stereotaxic head holder. Blood pressure and heart rate were monitored continuously with a Grass polygraph.† Rectal temperature was maintained at approximately 37°C.

Somatosensory evoked potentials were recorded from the right somatosensory cortex via a pair of bipolar platinum-iridium (90%/10%) electrodes with an interelectrode distance of 2 mm. The electrodes rested gently upon the dura mater exposed by burr holes, and recordings were made in response to bipolar supramaximal stimulation (approximately 1.0 V, 1 Hz, 0.4-msec duration) of the contralateral exposed sciatic nerve.‡ Throughout the experiment, a total of 200 successive SEP’s were amplified 1000 times with a preamplifier, summed on a signal averager, and recorded on Polaroid film for later measurement of the latency (msec) and amplitude (mV) of the initial positive wave.§ Three SEP recordings were obtained before injury at 30-minute intervals, and a mean latency and amplitude were calculated for each cat.

Local SCBF was determined using the H2 clearance technique, which allows for repeated measurements in the same animal.40 The SCBF electrode was constructed of 178-μ thick, triple Teflon-coated platinum-iridium (90%/10%) wire coated with nail polish.‖ The electrode tip was inserted into the dorsolateral funiculus of the spinal cord through a small hole in the meninges. The electrode was polarized to +350 mV with respect to a subcutaneous reference electrode. The reference electrode consisted of a silver/silver chloride pellet resting in a glass syringe plugged at the tip with agar and filled with a saturated KCl solution. Hydrogen was administered to the animals by directly bleeding the H2 gas into the intake line of a Harvard 607B respirator* for 3 minutes, with a resultant 1:1 mixture of room air and H2. As the H2 was taken up by the spinal tissue and oxidized at the electrode tip, a current was generated which was monitored on a Sargent-Welch polygraph.† Following H2 inspiration, the current declined as the tissue concentration of H2 fell. The initial 10-minute segment of the H2 decay curve was analyzed by taking measurements at 1-minute intervals starting at approximately 1 minute after the peak. The H2 decay curve values were approximated to a monoexponential function using a Hewlett-Packard 41C calculator.‡ A program calculated the correlation coefficient and a slope value; the latter was multiplied by 100 to give the SCBF in ml/100 gm/min. Decay curves with correlation coefficients of less than 0.990 were discarded. Less noisy H2 decay curves were obtained by thinly coating the spinal electrode with nail polish and by daily snipping of the tip to expose fresh wire. Three preinjury measurements were made at 30-minute intervals, and a mean preinjury SCBF value was determined for each cat. The electrode was removed just prior to contusion injury.

Blood gases (pO2 and pCO2) and pH were measured in some animals just before and at different times after H2 inhalation and were found to remain stable at normal levels throughout the blood flow measurement period. Spinal cord contusion injury was produced at the exposed L-3 spinal segment as described previously,8,36 except that a 500 gm-cm injury force was used (50-gm weight dropped 10 cm). Immediately following the contusion, the SCBF electrode was reinserted into the dorsolateral funiculus at the injury site, and blood

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* Kopf 1780 spinal unit manufactured by David Kopf Instruments, 7324 Elmo Street, Tujunga, California.
† Grass polygraph manufactured by Grass Instrument Co., 101 Old Colony Avenue, Quincy, Massachusetts.
‡ Grass S8 stimulator manufactured by Grass Instrument Co., 101 Old Colony Avenue, Quincy, Massachusetts.
§ Preamplifier, Model DAM-5A, manufactured by W-P Instruments, Inc., P.O. Box 3110, New Haven, Connecticut; 1550 signal averager manufactured by Tracor Northern, 2551 West Beltline Highway, Middleton, Wisconsin.
† Model 3001 polarograph manufactured by Sargent-Welch Co., 7300 North Linder Avenue, Skokie, Illinois.
‡ Calculator, Model 41C, manufactured by Hewlett-Packard Co., 1501 Page Mill Road, Palo Alto, California.

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TABLE 1
Mean arterial pressure in vehicle- and MPSS-treated spinal cord-injured cats*

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Cats</th>
<th>Mean Arterial Pressure (mm Hg)</th>
<th>Preinjury</th>
<th>30 Mins</th>
<th>1 Hr</th>
<th>2 Hrs</th>
<th>3 Hrs</th>
<th>4 Hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>vehicle</td>
<td>8</td>
<td>129.4 ± 6.5</td>
<td>108.8 ± 4.8</td>
<td>109.8 ± 4.0</td>
<td>107.7 ± 5.2</td>
<td>107.9 ± 5.7</td>
<td>102.3 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>15 mg/kg</td>
<td>5</td>
<td>132.6 ± 8.2</td>
<td>105.3 ± 11.5</td>
<td>99.7 ± 7.5</td>
<td>102.0 ± 5.1</td>
<td>96.3 ± 4.1</td>
<td>104.3 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>4</td>
<td>122.0 ± 7.5</td>
<td>90.8 ± 9.6</td>
<td>78.8 ± 11.0†</td>
<td>89.6 ± 11.9</td>
<td>95.0 ± 7.9</td>
<td>94.2 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>60 mg/kg</td>
<td>4</td>
<td>113.1 ± 5.4</td>
<td>87.1 ± 6.0</td>
<td>82.1 ± 1.4†</td>
<td>86.7 ± 3.9†</td>
<td>86.3 ± 5.7</td>
<td>87.9 ± 5.2</td>
<td></td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means. MPSS = methylprednisolone sodium succinate.
† Using the two-tailed t-test, these values were significantly different (p < 0.05) compared to those in the vehicle-treated cats.

flow determinations were made at 10 and 30 minutes, and at 1, 2, 3, and 4 hours postinjury.

At 30 minutes after injury, just before the 30-minute SCBF determination (except where described differently), each animal received a single bolus injection of 15, 30, or 60 mg/kg MPSS (Solu-Medrol sterile powder or Solu-Medrol vehicle) into the cephalic vein. The overall injection volume was kept constant by flushing the venous cannula with 1 to 2 ml of saline.

Results
Effects at 30 Minutes Postinjury

Figure 1 displays the time course of the changes in SCBF in the white matter of the 21 cats that received either the vehicle only or 15, 30, or 60 mg/kg MPSS as an intravenous bolus at 30 minutes after injury. The mean preinjury and the 10-minute postinjury SCBF values for all groups were combined since they were not significantly different from one another. The mean preinjury SCBF was 12.29 ml/100 gm/min, which is in good agreement with cat white matter SCBF values obtained by others using the H2 clearance method.39,41-43 At 10 minutes after injury, the mean SCBF for all four groups together was increased slightly. In the vehicle-treated group, there was a progressive fall in SCBF which was apparent at 30 minutes and became significant (p < 0.05 by t-test) by 2 hours after injury. By 4 hours after contusion, white matter blood flow within the contused spinal segment had fallen to 7.71 ml/100 gm/min, or to 62.7% of the mean preinjury level.

In contrast, administration of 30-mg/kg MPSS at 30 minutes after injury, just prior to the 30-minute SCBF determination, prevented the development of white matter ischemia. The mean SCBF in this group fell slightly at 30 minutes postinjury, but by 1 hour the SCBF had returned to, and at 3 and 4 hours had exceeded, the preinjury level (Fig. 1). The mean SCBF's in the animals receiving vehicle and in those treated with 30 mg/kg MPSS were significantly different from each other (p < 0.05 by t-test) at 3 and 4 hours after injury. The 15-mg/kg MPSS dose given at 30 minutes after injury partially prevented the fall in SCBF, but not as well as did the 30-mg/kg dose. However, the decline in SCBF from the preinjury level never became significant in the 15 mg/kg-treated cats over the course of the experiment. Interestingly, the 60-mg/kg MPSS dose was ineffective in supporting SCBF. The postinjury SCBF values for the 60 mg/kg-treated group were only about 1 ml/100 gm/min higher than the vehicle curve over the course of the experiment, and in essence the development of the ischemia was parallel in the two groups.

Table 1 gives the mean arterial pressures (MAP's) in the four groups of animals just prior to each SCBF determination shown in Fig. 1. As reported previously,26 the immediate effect of contusion injury to the cat lumbar spinal cord was a marked but transient increase in MAP, followed by a prolonged period of hypotension (a decrease of 20 to 25 mm Hg) in vehicle-treated animals. The 15-mg/kg MPSS dose given at 30 minutes after injury did not affect the fall in MAP, while the 30- and 60-mg/kg doses significantly intensified hypotension at certain times. However, only in the 30-mg/kg MPSS-treated group at 1 hour did the average MAP fall below 80 mm Hg. The maintenance of white matter SCBF at normal preinjury levels, even in the presence

Fig. 1. Mean (± standard error) blood flow in the dorso-lateral funiculus of the L-3 spinal segment before and after a 500 gm-cm contusion injury (C). Animals received either vehicle or varying doses of methylprednisolone sodium succinate as a single intravenous bolus at 30 minutes (arrow) after injury. N = number of cats.

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of a significantly greater postinjury hypotension, is consistent with the recently reported finding that when sympathetic function is intact, white matter SCBF is unaffected by changes in MAP over at least an 80- to 160-mm Hg range.41

It should be mentioned that in none of the vehicle- or MPSS-treated animals did SEP conduction, which was immediately and totally lost at the time of contusion, return over the course of the postinjury experimental period. While this finding is perhaps inconsistent with the greater incidence of SEP recovery observed in an earlier study that examined the effects of 15- and 30-mg/kg doses given 45 minutes after spinal cord injury in the cat,42 the disparity may be explainable by the fact that the other investigators employed a lesser injury force (400 gm-cm). In any event, the significant preservation of white matter SCBF as an effect of the 30-mg/kg dose can reasonably be expected to facilitate eventual functional recovery. This contention is based upon repeatedly demonstrated correlations between maintenance of white matter SCBF and either acute SEP return7,14-43 or voluntary functional recovery.17,18

Effect at 1½ or 4½ Hours Postinjury

Following the first series of experiments described above, in which a 30-mg/kg dose of MPSS given intravenously at 30 minutes after injury was found to optimally prevent the development of posttraumatic spinal white matter ischemia, it was decided to test the ability of this dose to restore SCBF after it had begun to fall. One set of four cats received the 30-mg/kg dose just prior to SCBF measurement 1½ hours after injury. Three of the four cats showed a measurable increase in SCBF. This constituted a small net increase in mean SCBF for the four cats of 6.88 to 7.75 ml/100 gm/min at 1½ hours compared to the 1-hour mean values, for an increase of only 12.7% (Fig. 2). This rather slight improvement was transient, however, being totally gone by the 3-hour postinjury measurement.

A 60-mg/kg intravenous dose of MPSS was given to another two animals (not shown in Fig. 2) just before SCBF measurement 1½ hours after injury. In one cat, SCBF increased by 1.3 ml/100 gm/min over the value at 1 hour, while in the other it decreased by about the same amount. Thus, increasing the dose given at 1½ hours did not result in a greater effect than seen with the 30-mg/kg dose given at that time.

In another three animals (from the vehicle group in the first experiment), the 30-mg/kg MPSS dose was not given until 4½ hours after injury (Fig. 2). The SCBF in these cats was unaffected over the subsequent 30-minute measurement period.

Discussion

These results confirm that the early administration of a single large intravenous dose of the glucocorticoid MPSS to cats after spinal cord contusion injury can prevent the development of posttraumatic spinal white matter ischemia. As also shown by others using H2 clearance methodology for recording sequential changes in SCBF,4 a 15-mg/kg MPSS dose given after injury, but before the onset of the delayed decline in SCBF, is partially effective in supporting SCBF. On the other hand, a 30-mg/kg dose completely prevents the development of ischemia over the first 4 hours after contusion.

In the present study, the dose-response relationship for the maintenance of white matter SCBF after injury is identical to that reported earlier, demonstrating that MPSS, given at 30 minutes after injury, can prevent an ischemia-related rise in spinal lactic acid levels, presumably due to an improved SCBF.8 However, in that instance the attenuation of the spinal tissue lactic acidosis was fairly transient, with a delayed rise in lactate levels occurring within 2 hours after injection of MPSS. This is in comparison to the prolonged support of white matter SCBF presently observed. The most likely explanation for this difference in duration of action concerns the probable differing rates of methylprednisolone elimination from white versus gray matter. In all probability, the primary site of lactic acid accumulation in the injured spinal cord is the gray matter. A biphasic kinetic pattern for methylprednisolone elimination from the injured cord9 suggests that it is eliminated from white matter much more slowly than from gray matter. The early rapid phase of methylprednisolone elimination from injured spinal cord has been shown to parallel the secondary increase in postinjury spinal lactic acid.8 In contrast, the more prolonged beneficial effect of MPSS on white matter SCBF may parallel the slower phase of this agent’s elimination in the spinal cord, in which the half-life is approximately 6 hours.9 This interpretation.

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is supported by the fact that, at least in normal cord, white matter SCBF is only about one-fifth to one-third that observed in gray matter.\(^1\),\(^2\),\(^28\),\(^29\),\(^37\)

In addition to the finding that large doses of MPSS are required to maintain blood flow to the injured spinal cord segment, similar doses have been shown to significantly decrease injury-induced oxygen free radical-generated spinal cord lipid peroxidation.\(^4\) While this effect could conceivably be secondary to the improved SCBF, a direct anti-oxidant capability of MPSS has also been clearly demonstrated.\(^5\),\(^25\),\(^38\) Moreover, equivalent intravenous doses of this steroid have been shown to directly enhance the excitability of normal cat spinal motor neurons.\(^7\),\(^24\) If this action is also relevant to injured spinal neurons, it may be a contributing factor in the recovery of impulse conduction after injury, a phenomenon observed by others.\(^42\)

The view that each of these effects may be potentially therapeutic in spinal cord injury, and in central nervous system (CNS) trauma in general, is suggested by the fact that they conveniently occur in the same dose range, with a 30-mg/kg intravenous dose appearing to be optimal for their production. Indeed, initial treatment of spinal cord-injured cats\(^14\),\(^33\) or monkeys\(^23\) with roughly equivalent doses of MPSS has been shown to result in a significantly facilitated neurological recovery.

The results of the present study also reveal a biphasic dose-response relationship for the effect of MPSS on SCBF. Although an MPSS dose in the range of 15 to 30 mg/kg can prevent the progressive development of posttraumatic spinal white matter ischemia, the administration of a higher (60 mg/kg) intravenous dose is similar to vehicle in its inability to maintain white matter SCBF. With a 60-mg/kg dose of MPSS, the rate of ischemia development following injury is essentially equal to that observed in vehicle-treated animals. A biphasic dose-response relationship has also been demonstrated in connection with the ability of MPSS to: 1) attenuate postinjury spinal cord lactic acidosis;\(^8\) 2) decrease \textit{in vitro}\(^5\),\(^25\) and \textit{in vivo}\(^28\) injury-induced spinal lipid peroxidation; and 3) enhance the excitability of normal spinal motor neurons.\(^7\),\(^24\) Thus, while large doses are needed to exert these supposedly beneficial actions on the injured CNS, these actions occur in a fairly narrow effective dose range. Therefore, caution is required so that the use of too large a dose will not result in a loss of each effect.

While the present study has confirmed that a 30-mg/kg intravenous dose of MPSS, when administered early after spinal cord injury, can prevent the development of white matter ischemia, its ability to reverse the fall in SCBF once it has occurred is limited, and within a short time is completely lost (Fig. 2). One possible explanation for this finding is based upon the fact that spinal tissue uptake of the steroid decreases with time after contusion injury. For instance, the spinal uptake of methylprednisolone from a 30-mg/kg intravenous dose has been found to be half as great when given at 1 hour or longer after injury than when given at 30 minutes.\(^9\) To test this possible reason for the lack of effect, a 60-mg/kg dose was given at 1\(^\frac{1}{2}\) hours after trauma to two cats to see if a larger MPSS dose, which would be expected to result in higher tissue levels,\(^7\) could better restore white matter SCBF toward normal. As noted in the Results, one of the cats showed a slight improvement in SCBF, while in the other SCBF continued to decline. Thus, it would appear that tissue steroid uptake, while obviously important, may not be the primary basis for the inability of MPSS to reverse the ischemia.

An alternative explanation is that the pathophysiological changes that underlie the posttraumatic white matter ischemia are, for the most part, irreversible. Perhaps the most significant occurrence in this regard might be extensive free radical-induced lipid peroxidation.\(^13\),\(^15\),\(^27\) Biochemical evidence of significant lipid peroxidation within the spinal cord has been demonstrated to occur within an hour after severe spinal cord contusion.\(^11\),\(^14\),\(^30\) One can ascertain that the ischemic hypoxia and associated oxygen-derived free radical generation that lead to lipid peroxidation probably begin in the central gray matter. Gray matter blood flow is decreased first after injury,\(^12\) due to mechanical damage to its more dense microvasculature, to excessive generation of thromboxanes,\(^29\) and possibly to release of vasoactive neurotransmitters such as serotonin.\(^10\) The lipid peroxidative reactions that are in turn promoted by iron complexes\(^2\) provided by gray matter petechial hemorrhages are self-perpetuating and gradually would be expected to spread to the circumferential white matter tracts. There they would irreversibly damage myelin and axons directly, as well as indirectly due to ischemia secondary to lipid peroxidative microvascular destruction.

Methylprednisolone, if given early, may stop this sequence of events at the gray matter level through multiple actions, perhaps including: 1) a direct antioxidant effect,\(^6\),\(^25\),\(^38\) 2) an inhibition of arachidonic acid release,\(^34\) thereby preventing thromboxane formation; 3) a decrease in the vascular responsiveness to vasoactive neurotransmitters;\(^7\) and 4) a direct vasodilator action.\(^22\) (See the review by Hall and Braughler\(^27\) for a more in-depth discussion of this latter topic.) However, as time passes and more and more peroxidative microvascular damage occurs, the efficacy of the glucocorticoid in manifesting these actions becomes progressively less.

Additional support for a critical role of vascular lipid peroxidation in the development of posttraumatic white matter ischemia is provided by the identical dose-response relationship for the effect of MPSS on post-injury lipid peroxidation\(^27\) and on the development of white matter ischemia. Specifically, the 30-mg/kg dose of MPSS is associated with a decrease in lipid peroxidation and the prevention of ischemia, while the 60-mg/kg dose does not prevent lipid peroxidation or
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ischemia. A definitive demonstration of the relationship between blood flow and microvascular integrity has been provided by Anderson, et al., who have shown that high-dose MPSS administered early after cat spinal cord compression injury acts to improve cord energy metabolism and preserve both the gray and white matter microvasculature.

As a final point in this regard, the opiate receptor antagonist, naloxone, has also been shown to prevent the development of post-contusion spinal white matter ischemia and to promote sensorimotor recovery in spinal cord-injured cats. Interestingly, this beneficial action of naloxone requires early treatment with large nonselective doses (2 to 10 mg/kg), which suggests that some mechanism other than opiate receptor blockade (that is, possibly a direct membrane action) may be responsible for the therapeutic effect. Indeed, high concentrations (0.5 mM or higher) of naloxone have been shown to inhibit iron-catalyzed peroxidation in artificial liposomes. Thus, this indirect evidence concerning possible lipid-protecting actions of naloxone in relation to CNS injury further suggests a role of lipid peroxidation in the evolution of posttraumatic CNS ischemia and tissue destruction.

In summary, a single large (30-mg/kg) intravenous dose of MPSS can significantly prevent the development of posttraumatic spinal cord white matter ischemia. On the other hand, MPSS administration does not appear to greatly reverse the decrease in SCBF once it has occurred.

References


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