Evaluation of an intensive methylprednisolone sodium succinate dosing regimen in experimental spinal cord injury

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Beginning 30 minutes after compression trauma of the upper lumbar (L-2) spinal cord, cats were treated with either a high-dose regimen of methylprednisolone (MP) administered as the sodium salt of the 21-succinate ester (Solu-Medrol sterile powder) or the MP vehicle. Animals were randomly assigned to either treatment group (10 cats per group), and all personnel were blind as to which animals received vehicle or drug. The intensive 48-hour dosing regimen was designed to maintain therapeutic tissue levels of MP and consisted of an initial 30 mg/kg intravenous bolus of MP; 2 and 6 hours later additional 15 mg/kg MP doses were administered by intravenous bolus. Immediately following the bolus given at 6 hours, a continuous MP infusion of 2.5 mg/kg/hr was started. The infusion was stopped abruptly at 48 hours with no dose tapering. Animals in the vehicle group received an equivalent volume of MP vehicle. The total MP dose administered over 48 hours was 165 mg/kg. Animals were evaluated weekly for neurological recovery based upon a 12-point functional scale which assessed general mobility, running, and stair-climbing. Mean recovery scores at 1 month after injury (± standard error of the mean) were: vehicle group (seven cats) 3.7 ± 0.9, and MP group (10 cats) 8.7 ± 0.2; (p < 0.001). Histological evaluation of the spinal cords revealed a strong negative correlation between neurological recovery and size of the spinal cord cavity at 1 month (r = -0.88). Three of 10 animals in the vehicle group became ill and had to be dropped from the study, whereas all of the 10 MP-treated animals survived in excellent health. The results demonstrate the therapeutic effectiveness and low incidence of side effects associated with an intensive MP dose regimen for treatment of experimental spinal cord injury.

KEY WORDS • spinal cord injury • methylprednisolone • cat

A VARIETY of potentially beneficial actions of methylprednisolone (MP) on the injured central nervous system (CNS) have been reported by a number of laboratories. In experimental models of spinal cord injury, MP has prevented posttraumatic spinal cord ischemia,18,24 improved energy metabolism,7,9 restored extracellular calcium,7,24 inhibited tissue lipid peroxidation,1,15,16 improved nerve impulse conduction,5,17,24 and blocked the release of free fatty acids, eicosanoids, and the loss of cholesterol from injured spinal cord tissue.2,11 In all cases where the MP dose-response curve has been examined, the optimal intravenous dose of MP to achieve these varied actions was on the order of 30 mg/kg. Steroid doses such as these have recently been termed "CNS injury" doses.8 Tissue pharmacokinetic and pharmacodynamic studies of MP and its effects in the injured spinal cord have indicated that its actions are dependent upon critical levels of drug in the injured tissue.5,7,9,10,18 Furthermore, these studies have predicted that intensive maintenance dosing would be required to assure maintenance of optimal tissue concentrations.

Based upon acute pharmacokinetic and pharmacodynamic studies of MP in the injured spinal cord, a dosing regimen was proposed that was designed to rapidly achieve and maintain optimal therapeutic levels of the steroid in CNS tissue. The development of this regimen was founded solely upon the pharmacology of MP at high doses and was not influenced by classic glucocorticoid pharmacology. Indeed, the beneficial actions of MP on the injured CNS are believed to be unrelated to its glucocorticoid (hormonal) activity.5,17 This hypothesis is supported in part by in vitro studies demonstrating acute and direct antioxidant-like and
membrane-protective activities of high concentrations of glucocorticoids, including MP.\(^{3,4,23}\)

Questions have been raised relating to the safety and efficacy of intensive therapy with large doses of MP. In the present study, both the safety and the efficacy of an intensive short-term dosing regimen of MP sodium succinate (MPSS) are reported in an experimental model of spinal cord injury.

**Materials and Methods**

**Operative and Injury Procedures**

Twenty adult mongrel cats, ranging in weight from 2 to 4 kg, were used for this study. All cats were anesthetized with intraperitoneal pentobarbital sodium (30 mg/kg) and were intubated. Polyethylene cannulas were inserted into a femoral artery and vein. Systemic arterial blood pressure was monitored via the arterial catheter, which was also used to obtain arterial samples for blood gas and pH analysis. Drugs were administered through the venous cannula. Muscle paralysis was achieved with intravenous succinylcholine chloride (1 mg/kg), and the animals were ventilated with positive pressure using a mixture of room air, 95% O\(_2\), and 5% CO\(_2\). This gas mixture was necessary to simultaneously maintain the desired arterial partial pressures of both O\(_2\) and CO\(_2\). Blood gas levels were controlled by adjusting the respiratory minute volume and/or the flow rate of the gas mixture.

In all animals, the vertebral column was exposed in the upper lumbar region (at L-2) and a laminectomy was performed. Trauma was induced by placing a 170-gm weight extradurally on the spinal cord for 5 minutes.\(^{2,22}\) The wound was closed in layers, and the cats were placed in a veterinary intensive care unit for 48 hours. All cats were given 100,000-U intramuscular doses of procaine penicillin G in dihydrostreptomycin solution daily for the first 3 postoperative days.

**Drug Treatment and Animal Evaluation**

After injury, the cats were randomly assigned to two groups of 10 animals each. One group received MP administered as the sodium salt of the 21-succinate ester (Solu-Medrol sterile powder, MPSS) and the other received the MPSS vehicle. All study personnel were blind as to the nature of the treatment. Drug or vehicle was freshly prepared in coded vials and delivered to the operating room immediately prior to use. Beginning 30 minutes after injury, the cats received an intravenous (IV) 30-mg/kg bolus of either vehicle or MPSS. Two hours later, the animals received a second IV 15-mg/kg bolus of either vehicle or MPSS. Four hours later, the animals received a third IV 15-mg/kg bolus of either vehicle or MPSS. At this time, a continuous IV 2.5-mg/kg/hr infusion of either vehicle or MPSS was started and continued for the remaining 42 hours. The total dose over 48 hours was 165 mg/kg (Table 1).

Originally, 10 cats were in the vehicle-treated group. However, during the course of the experiment, three became ill, which significantly affected their recovery and necessitated their removal from the study. All cats were allowed to recover for 4 weeks and their functional recovery was evaluated on a weekly basis. Our neurological evaluation procedure was based on observing and rating the mobility of a freely moving animal in various controlled situations. The cats were rated according to the criteria set out in Table 2.

Each animal was assigned a score in each category, and the total score was the value used as an index of the degree of neurological functional recovery (recovery index). Twelve was the highest obtainable score and denotes normalcy (preinjury function).

**Morphological Investigation Methods**

Cats were sacrificed 30 days following compression injury by intra-aortic perfusion fixation following administration of intraperitoneal sodium pentobarbital (30 mg/kg).\(^{20}\) The perfusion was initiated with warm (37°C) lactated Ringer’s solution that contained 2 cc (10,000 U) sodium heparin and 1% sodium nitrate, and continued until there was clear return from the right atrium. This was followed by an infusion of 3 liters of warm 10% formalin. The dura was carefully removed and the spinal cord was divided transversely into two pieces. Each piece of tissue was processed in a routine fashion and embedded in paraffin. It is recognized that
shrinkage of tissue occurs in paraffin-embedded material as in other embedding techniques. Care was taken to treat all tissue similarly so that shrinkage affected all tissue equally. The tissue was sectioned serially at 10 μ, beginning at the block face at the center of the lesion. Each group of sections was mounted on glass slides and stained with either hematoxylin and eosin (H & E) or Luxol fast blue-periodic acid-Schiff (LFB-PAS).

Every 10th section of tissue was photographed on a Leitz Dialux photomicroscope, using a ×1 objective. The negatives were enlarged to a final magnification of approximately ×25. The perimeter of the spinal cord sections and cavities were digitized on photographs using a Leitz Videoplan semiautomatic image analyzer. The maximum cross-sectional area of the cavities (% area = area cavity/area cord × 100) was determined with the aid of programmed calculation on the Videoplan analyzer.

**Statistical Analysis**

Difference among the groups was assessed by unpaired Students’ t-test. The minimum level of significance was set at 0.05. The correlation coefficient (r) was also computed to compare the recovery index with the morphometric parameters of the percent of spinal cord cross-sectional area taken up by the cavity.

**Results**

Following a compression injury of 170 gm/5 min, there was progressive recovery of function in both groups of cats over the 4-week postinjury period (Fig. 1). However, the MPSS-treated cats exhibited a significantly earlier and more complete recovery than the vehicle-treated cats. By the 2nd week of recovery, the MPSS-treated cats demonstrated a significantly higher degree of neurological function than the vehicle-treated cats. By the 4th week, the injured, vehicle-treated cats had recovered 31% of their neurological function (mean score 3.7 out of a maximum of 12), whereas the MPSS-treated cats had recovered 72% of their normal function (mean score 8.7 out of 12).

The correlation coefficient between the recovery index and the size of the cavity in the spinal cord at 4 weeks after injury was −0.88. This excellent correlation indicates a strong negative relationship between the degree of recovery and the size of the cavity in the spinal cord (that is, the greater the recovery the smaller the cavity size and vice versa).

Three of 10 vehicle-treated cats became critically ill during the course of the study and were excluded because their demeanor and ability to perform motor function tests were adversely affected. In contrast, none of 10 MPSS-treated animals became ill. Although no formal rating was made, subsequent evaluation of careful notes maintained on each animal in the study indicated that the general health and demeanor of MPSS-treated animals were far superior to those of vehicle-treated animals. No evidence of gastrointestinal bleeding or pathological immunosuppression was observed in either the MPSS- or the vehicle-treated group.

**Discussion**

Previous studies have demonstrated that MP is effective in reducing the neurological deficit and tissue loss in cats undergoing compression trauma of the spinal cord. In the present study, we used considerably larger doses for a shorter period of time and confirmed the effectiveness of MP in protecting against the effects of traumatic injury to the spinal cord in experimental animals. While high doses of MP such as these may precipitate unwanted side effects, we observed no toxicity that might otherwise limit the clinical utility of the high-dose regimen described in this report. In all probability, glucocorticoid-related side effects were limited due to the short duration (48 hours) of treatment with these high doses. The various mechanisms by which high doses of MP affect the injured CNS are probably largely unrelated to its glucocorticoid, receptor-mediated activity. This has been suggested for MP by the need for large doses and frequent maintenance dose administration in order to sustain its therapeutic effects.

The intensive “CNS injury” MP dosing regimen described in this report was specifically designed to rapidly achieve and maintain therapeutic tissue levels of drug over a short period of time. The regimen was based upon a rational pharmacological evaluation of the actions of high MP doses on the injured CNS. In earlier studies from a number of laboratories, less intense MP regimens have been reported to promote functional recovery in animals following experimental spinal cord trauma. In all probability, in the...
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carefully controlled experimental situation where treatment is initiated within 30 minutes following trauma, less optimal and less intense MP regimens may offer some protective influence. However, a lack of therapeutic effect has also been reported for very low doses of glucocorticoid in experimentally injured animals. In the clinical situation, where even under the best of circumstances treatment may be delayed for 1 or 2 hours following injury, an optimal MP dose regimen would be critical. It is difficult in retrospect to compare the efficacy of the MP regimen reported here with the results from previously reported studies of MP in chronic experimental spinal cord injury studies. It is clear, however, that the intensive MP dosing regimen described in this study was not only highly effective at promoting functional recovery following severe experimental spinal cord injury, but was also without untoward effects.

Acknowledgment

The authors thank Evangelyn Sue Green for her technical expertise in the conduct of these studies.

References


Manuscript received August 12, 1986. Accepted in final form January 12, 1987.
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