Correlation of methylprednisolone levels in cat spinal cord with its effects on (Na\(^+\) + K\(^+\))-ATPase, lipid peroxidation, and alpha motor neuron function

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Large intravenous doses of methylprednisolone sodium succinate are associated with biochemical and electrophysiological effects in the cat spinal cord which may be of therapeutic value in the treatment of spinal cord injury. The potentially beneficial effects of large doses of the glucocorticoid include: 1) an enhancement of spinal cord (Na\(^+\) + K\(^+\))-ATPase activity; 2) an attenuation of lipid peroxide formation; 3) a hyperpolarization of motor neuron resting membrane potentials; and 4) an accelerated impulse conduction along the myelinated portion of the motor axon. Each of these is apparent with spinal cord tissue levels of methylprednisolone around 1.3 \(\mu\)g/gm wet weight, which are rapidly obtained following a single intravenous dose of 30 mg/kg. The half-life of methylprednisolone in cat spinal cord following a single intravenous administration, as well as the duration of its pharmacological actions, is roughly 3 hours. The data suggest that, in order to be of therapeutic value in the treatment of acute spinal cord trauma, early intervention with high-dose intravenous methylprednisolone (30 to 40 mg/kg) is necessary, followed by intravenous maintenance dosing of 15 to 20 mg/kg every 2 to 3 hours. The rationale and duration for this regimen are discussed.

KEY WORDS • methylprednisolone • spinal cord injury • steroid

A number of animal studies have demonstrated an improved functional recovery following blunt spinal cord trauma as a result of intensive high-dose glucocorticoid (such as methylprednisolone and dexamethasone) treatment.\(^1\)\(^,\)\(^2\)\(^,\)\(^6\)\(^,\)\(^9\)\(^,\)\(^11\)\(^,\)\(^16\)\(^,\)\(^19\) Although these investigations have varied in terms of the particular animal species used, the type of injury, and the specific dose of glucocorticoid and treatment regimen, common to each of these studies has been the early initiation of therapy and the use of large doses of glucocorticoid.

The mechanism(s) responsible for the improved sensorimotor recovery following the intensive treatment of spinal cord trauma with glucocorticoids remains unknown. Furthermore, the optimal dose of glucocorticoid is not known, nor has the best therapeutic regimen been developed. Ducker and Hamit\(^6\) postulated that intensive glucocorticoid dosing acts to maintain microvascular integrity and reduce spinal cord edema. On the other hand, Lewin, et al.,\(^16\) found that edema formation in the acutely injured spinal cord is not significantly affected by glucocorticoid administration, despite an improved functional recovery. Rather, these investigators have shown that glucocorticoid treatment reduces potassium loss from the injured cord, suggesting a preservation of neuronal structure. Other investigators\(^10\)\(^,\)\(^18\) have demonstrated a significant anatomical preservation as a result of the early initiation of glucocorticoid treatment following blunt spinal cord trauma.

Demopoulos, et al.,\(^7\)\(^,\)\(^8\) have suggested that a principal mode of glucocorticoid action on the traumatized spinal cord may be the attenuation of free radical reactions precipitated by injury. Free radical formation resulting from ischemia and catalyzed by hematin compounds derived from extravasated blood in the injured cord is believed to lead to the peroxidation of phospholipids in myelin, and neuronal and microvascular membranes. If unchecked, such lipid peroxidation may lead to a decrease in the activities of certain key neuronal enzymes such as (Na\(^+\) + K\(^+\))-adenosine triphosphatase (ATPase),\(^4\) which is largely responsible for the maintenance of the neuronal resting membrane potential.

Recent studies from our laboratories have shown that single large intravenous doses of methylprednis-
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Methylprednisolone (30 mg/kg) can inhibit lipid peroxide formation in spinal cord homogenates, enhance synaptosomal (Na⁺ + K⁺)-ATPase activity, and produce a resting hyperpolarization and shortened antidromic action potential latency of spinal cord motor neurons. Since each of these effects may constitute important therapeutic actions of the glucocorticoid in the injured spinal cord, the present investigation was undertaken to study the tissue pharmacokinetics of intravenous methylprednisolone in the cat lumbar spinal cord. The purpose of such an investigation was to provide a necessary correlate for the previously described glucocorticoid effects on lipid peroxidation, (Na⁺ + K⁺)-ATPase, and neuronal function such that a therapeutic regimen appropriate for the production and maintenance of these effects in the injured spinal cord could be developed.

Materials and Methods

Animal Preparation and Steroid Administration

Adult cats of either sex, weighing 1.8 to 4.0 kg, were anesthetized with intravenous α-chloralose (80 mg/kg), and the lumbar spinal cord from L-5 to L-2 was exposed via dorsal laminectomy. Animals were paralyzed intravenously with gallamine triethiodide (3 mg/kg) during surgery and were placed on positive-pressure ventilation with room air. Methylprednisolone sodium succinate (Solu-Medrol) was administered through the brachial vein in a single dose of either 15, 30, 60, or 90 mg/kg. At the time of cord removal, the dura was opened and the lumbar spinal cord from L-4 to L-2 was rapidly removed, frozen in liquid nitrogen, and stored at -70°C until assay.

Determination of (Na⁺ + K⁺)-ATPase Activity

Synaptosomal (Na⁺ + K⁺)-ATPase activity in a section of the frozen spinal cord was prepared and assayed as described previously. Synaptosomes were prepared by homogenizing frozen spinal cord (400 to 500 mg) at 4°C in 9 volumes of 100 mM Tris-HCl (pH 7.6) containing 330 mM sucrose. The homogenate was centrifuged at 1100 G for 10 minutes and the resulting supernatant fraction was centrifuged at 18,000 G for 15 minutes. The synaptosomal pellet was resuspended in fresh homogenizing buffer and centrifuged again at 18,000 G for 15 minutes. The resulting pellet was resuspended in 1 ml of homogenizing buffer and was assayed immediately. The (Na⁺ + K⁺)-ATPase assay was placed for 15 minutes at 37°C in 100 mM imidazole buffer (pH 7.0) containing 6 mM vanadium-free ATP, 6 mM MgCl₂, 100 mM NaCl, 20 mM KCl, and 0.04 to 0.08 mg synaptosomal protein/ml. Inorganic phosphate released from ATP was determined by the method of Lowry and Lopez. The value of (Na⁺ + K⁺)-ATPase was calculated from the difference between that activity in the absence (total - ATPase) and presence (Mg²⁺ - ATPase) of 1 mM ouabain. Protein was determined by the method of Lowry, et al. Activity of (Na⁺ + K⁺)-ATPase is expressed as nmoles Pi/mg protein/min.

Determination of Lipid Peroxide Formation

Lipid peroxide formation during a 1-hour incubation of spinal cord homogenates at 37°C was estimated using the thiobarbituric acid test for malondialdehyde as described. Frozen spinal cord (200 mg) was homogenized in 1 ml of 100 mM Na phosphate buffer (pH 7.0), and 0.5 ml aliquots were incubated in duplicate at 37°C for 1 hour. After incubation, 0.5 ml of 20% trichloroacetic acid was added to each sample, which was then centrifuged at 3000 G for 15 minutes. The resulting supernatant fraction was then diluted 1:1 with 10% trichloroacetic acid containing 0.67% thiobarbituric acid, heated in a boiling water bath for 10 minutes, then cooled, and the absorbance read at 530 nm. Malondialdehyde was quantitated on the basis of a molar extinction coefficient of 1.56 x 10⁵.

Methylprednisolone Assay

The methylprednisolone content of the spinal cord samples was measured by a method recently developed in our laboratories which will be described in detail elsewhere (McGinley, Braughler, and Hall, manuscript in preparation). Briefly, a sample of frozen spinal cord weighing approximately 400 mg was homogenized in 5 ml of ether:methylene chloride (60:40). The homogenate was centrifuged, then washed sequentially with 1 ml of 0.1 N NaOH, 0.1 N HCl and distilled H₂O. The organic phase was evaporated to dryness, reconstituted in 300 μl of methylene chloride:isopropanol (85:15), which also contained 500 ng of triamcinolone acetonide as an internal standard, and 100 μl was injected into the HPLC column. The chromatographic system consisted of a Varian 5020 HPLC, a CDC 111 integrator, a fixed wavelength detector (254 nm), and a bonded phase micropak NH₂ column.* The eluent used was methylene chloride:isopropanol (85:15) at a flow rate of 2 ml/min. Methylprednisolone was quantitated on the basis of the peak area ratio of triamcinolone acetonide to methylprednisolone.

Intracellular Recording Techniques

In other animals, conventional intracellular recording techniques were employed to measure motor neuron resting membrane potentials and antidromic action potential latencies following methylprednisolone administration. A random population of lumbar spinal motor neurons was sampled in unanesthetized acutely decerebrate (C-1 sectioned) cats. Each was briefly anesthetized with halothane and tracheoto-

* Chromatographic system manufactured by Varian Corp., Walnut Creek, California.
mixed. The spinal cord was transected at the C-1 level and the animal placed on positive-pressure ventilation with room air. Anesthesia was then discontinued. One carotid artery was cannulated for blood pressure recording and the other was ligated to produce cortical ischemia and thus insure unconsciousness.

A dorsal laminectomy exposed the spinal cord from the L-3 to the S-2 level. The L-6, L-7, and S-1 dorsal roots were sectioned unilaterally at their points of exit from the spinal column and reflected to the opposite side of the cord. The ipsilateral L-7 and S-1 ventral roots were also sectioned distally. The cats were placed in a prone position in a Kopf 1780 spinal unit.† A bilateral pneumothorax was made to reduce respiratory chest movements, and gallamine triethiodide was given (3 mg/kg intravenously plus supplemental doses as needed) to produce total neuromuscular paralysis. The skin edges of the back were retracted to form a basin that was filled with saline to a level just covering the spinal cord and then completely filled with mineral oil. The temperature of the bath was maintained at 37°C via radiant heat. The distally sectioned ventral roots were pulled up and placed on a pair of bipolar platinum-iridium stimulating electrodes above the saline level. An Ag-AgCl reference cell was placed down in the saline.

Glass microelectrodes with 4 to 10 mOhm tip resistances and filled with 2M potassium acetate were used for recording. The electrodes were advanced vertically using a hydraulic microdrive penetrating the spinal cord 1 mm lateral to the L-6, L-7, and S-1 dorsal root entry zones. Entry of an alpha motor neuron was signalled by a sudden negative shift in the DC-coupled oscilloscopic sweep and the appearance of a short-latency antidromic action potential. The 60 mg/kg dose, however, was also seen (data not shown).

Figure 1 shows the effects at 1 hour of single intravenous doses of methylprednisolone (15 to 90 mg/kg) on spinal cord synaptosomal (Na⁺ + K⁺)-ATPase activity, malonyldialdehyde formation, and the resting membrane potential and action potential latency of spinal motor neurons. Also shown are the tissue levels of methylprednisolone determined with each dose of steroid 1 hour after injection. The levels of methylprednisolone in the cat spinal cord following a single intravenous injection were increased with increasing doses of steroid. Tissue levels of 1.3 μg/gm wet tissue and more were associated with significant effects on spinal cord (Na⁺ + K⁺)-ATPase, lipid peroxide formation, and motor neuron function.

The (Na⁺ + K⁺)-ATPase activity was significantly increased in a dose-dependent manner as a function of the levels of methylprednisolone present in the spinal cord. The threshold dose for the increase in activity was in the 15 mg/kg-dose range. Doses higher than 15 mg/kg up to at least 60 mg/kg produced a progressively greater enhancement of enzyme activity.

Malonyldialdehyde formation in spinal cord homogenates was significantly reduced with a dose of 30 mg/kg. This dose corresponded to a methylprednisolone tissue level of 1.3 μg/gm wet tissue. Although the 60 mg/kg dose also reduced malonyldialdehyde formation to nearly the same extent as the 30 mg/kg dose, it was not shown to be statistically different from control (p < 0.1). On the contrary, a tissue concentration of 5 μg/gm wet tissue which was obtained with a 90 mg/kg dose tended to be associated with an enhancement of in vitro lipid peroxidation.

The 30 mg/kg dose of methylprednisolone produced a hyperpolarization of alpha motor neuron resting membrane potentials as measured within 1 hour of drug administration. In addition, this dose also significantly shortened the antidromic latency or the time from delivery of a stimulus to the myelinated motor axon to the beginning of the initial segment portion of the antidromic motor neuron action potential. Thus, the shortened latency represents an accelerated impulse conduction along the myelinated portion of the axon. The 60 mg/kg dose, however, was without significant effect on either parameter. Doses higher than 60 mg/kg were not examined.

Methylprednisolone Levels and Duration of Pharmacological Effects

The time course for the effects of a single intravenous 90 mg/kg dose of methylprednisolone on (Na⁺ + K⁺)-ATPase activity and the disappearance of the glucocorticoid from spinal cord tissue are shown in Fig. 2. The time course of the glucocorticoid-induced enhancement of (Na⁺ + K⁺)-ATPase activity approximated the levels of methylprednisolone in the spinal cord which displayed a half-time for tissue elimination of 3 hours. A similar time course for the effects of methylprednisolone on malonyldialdehyde formation was also seen (data not shown).

Discussion

The present study correlates the dose and spinal cord tissue levels of methylprednisolone with its effects on (Na⁺ + K⁺)-ATPase, lipid peroxide forma-
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Fig. 1. Graphs showing correlation of methylprednisolone levels in the spinal cord, with its effects on lipid peroxidation, (Na$^+$ + K$^+$)-ATPase, motor neuron resting membrane potentials, and antidromic action potential latencies. Cats prepared as described in Methods were given a single intravenous bolus injection of the dose of methylprednisolone sodium succinate indicated. One hour after the injection, a section of the lumbar spinal cord (L4-2) was removed, frozen in liquid N$_2$ (Panels A-C), and stored at -70°C until assayed for methylprednisolone content (Panel A), malonyldialdehyde formation (Panel B), or (Na$^+$ + K$^+$)-ATPase activity (Panel C) as described in Methods. In some animals (Panel D), alpha motor neuron resting membrane potentials (black circles) and antidromic action potential latencies (open circles) were determined within 1 hour following the administration of methylprednisolone as described in Methods. Values represent the mean ± standard error (SE) from the number of animals (Panels A-C) or motor neurons (number of animals in parenthesis, Panel D) indicated above the SE bar and are significantly different from control by the p value indicated below the SE bar (Student's t-test).

From the results presented here, it is clear that large intravenous doses of methylprednisolone can have profound effects on spinal cord biochemistry and electrophysiology which may be relevant to the more successful treatment of central nervous system (CNS) trauma with glucocorticoid. Spinal cord tissue concentrations of methylprednisolone around 1.3 μg/gm wet tissue occurred within 5 minutes of the intravenous administration of a 30 mg/kg dose of methylprednisolone and were associated with a decrease in malonyldialdehyde for-
gested by the work of a number of investigators. 4,
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of lipid peroxides and other free radical reaction
within the fatty acid component of myelin and neu-
related to an increase in the generation of free radicals
injury self-destruction process in the spinal cord is
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ATPase activity following a contusion injury, perhaps
there is a rapid reduction in spinal cord (Na § + K+)-ATP -
ed enzyme has been sug-
portant role in the maintenance of the neuronal trans-
zymes and ultimately to tissue breakdown. One such
in the destruction of key membrane-associated en-
ent of the neuronal membrane is believed to result
peroxidation, which would allow a preservation of
structural integrity and a support of membrane-bound
zyme activities. The results from the present study
are in agreement with this hypothesis and have pro-
vided a definition of the dose-response and time-
action characteristics of the potentially beneficial ac-
tions of methylprednisolone to decrease spinal lipid
peroxidation and to enhance (Na+ + K+)-ATPase
activity. Based upon the dose and tissue levels asso-
ciated with the glucocorticoid’s biochemical actions
described here, an intravenous methylprednisolone
dose of at least 30 mg/kg is required to produce
these positive effects.

In addition to the production of these biochemical
effects, the 30 mg/kg methylprednisolone dose has
also proved optimal for the generation of certain
spinal electrophysiological actions which would
clearly be beneficial in the injured cord. For instance,
the resting hyperpolarization of spinal motor neurons,
if indicative of a general high-dose glucocorticoid
effect on neuronal polarization, would counter the
tendency of damaged neurons to irreversibly depolar-
ize and thus to become inexcitable, as discussed by
Kobrine.14 Furthermore, the increase in spinal axonal
conduction velocity induced by high-dose methyl-
prednisolone would serve to facilitate white matter
impulse conduction in the injured spinal cord.

Although the results presented in this study dem-
strate that large intravenous doses of methylpred-
insolone are required to beneficially affect spinal cord
biochemistry and electrophysiology, it is perhaps crit-
tical to note that doses of 60 mg/kg and greater were
less effective than the 30 mg/kg dose. The 90 mg/kg
dose, in fact, was even found to enhance lipid perox-
idation under the conditions of the thiobarbituric acid
test. These studies suggest that the optimal methyl-
prednisolone dose for treating spinal cord trauma is
in the 30 mg/kg range.

The need for high doses of glucocorticoid in the
treatment of spinal cord trauma has been suggested
by the work of a number of laboratories. 1,2,3,6,9,11,16,19
The results presented here, however, argue in favor of
the use of even higher doses than those previously
examined. Furthermore, with the elimination half-
time of methylprednisolone from spinal cord tissue of
roughly 3 hours, multiple dosing with methylprednis-
olone every 2 or 3 hours would be necessary to
maintain tissue levels in the therapeutic range. The
current clinical use of glucocorticoids for the treat-
ment of spinal cord trauma employs doses far below
those giving effective tissue concentrations in the pres-
ent study. In addition, based upon our studies, a

Fig. 2. Graph showing the tissue half-life of methyl-
prednisolone in spinal cord tissue and the duration of its
effect on (Na+ + K+)-ATPase. Cats prepared as described
in Methods were given a single intravenous bolus of methyl-
prednisolone sodium succinate (90 mg/kg). At the times
indicated (5 minutes and 1, 4, and 24 hours) following the
injection, a section of spinal cord (L4-2) was removed,
frozen in liquid nitrogen, and stored at -70°C until assayed
for methylprednisolone content (black circles) or (Na+ +
K+)-ATPase activity (open circles) as described in Methods.
Values are mean ± standard error (SE) based on determin-
ations from the number of animals indicated above the
SE bar.
dosing interval for maintenance therapy of 4 to 6 hours would not be effective in maintaining tissue levels of methylprednisolone in the range necessary to decrease lipid peroxidation, support (Na\(^+\) + K\(^+\))-ATPase activity, and facilitate neuronal excitability and impulse conduction. The studies described here suggest that an intravenous loading dose of 30 to 40 mg/kg, followed by maintenance dosing of 15 to 20 mg/kg every 3 hours, would be effective in maintaining appropriate tissue concentrations of the drug.

The course of such treatment for spinal cord trauma remains empirical. Most studies employing lower doses of glucocorticoids in the treatment of experimental CNS trauma have continued treatment for periods of 7 to 14 days. In one study which examined the use of glucocorticoids in the treatment of spinal cord injury in monkeys, Green, et al.,\(^{11}\) reduced the dose of steroid stepwise in half on a daily basis for 5 days. Probably more important than the length of treatment, however, is the critical time period in which treatment is begun following a spinal cord injury. It has been suggested that damaging free radical reactions occur as a very early event following spinal cord trauma, and that anatomical degeneration and resulting permanent loss of function are the result of early damage.\(^{9}\) In fact, we have observed that lipid peroxide formation is increased within 5 minutes following a 400 gm-cm impact to the cat spinal cord (Plaster, Braughler, and Hall, unpublished observations). Therefore, initiation of high-dose glucocorticoid therapy at the earliest possible time following injury would seem to offer the best chance for clinical improvement.

With regard to potential side effects of intensive treatment with methylprednisolone, Novak, et al.,\(^{20}\) reported in a randomized double-blind study that single large intravenous doses (30 mg/kg) of methylprednisolone were safe for use. In another study, Woods, et al.,\(^{22}\) reported a low incidence of drug-related complications in renal transplant patients receiving 1 gm of intravenous methylprednisolone per day for 2 days with subsequent stepwise half reductions in the total daily dose for 5 days. Gastrointestinal hemorrhage may be the most commonly expected complication of intensive glucocorticoid therapy in patients with spinal cord injury. Green, et al.,\(^{11}\) and Conn and Blitzer,\(^{5}\) however, have suggested that gastrointestinal hemorrhage is associated more with stress following spinal cord injury than steroid administration.

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