The effects of methylprednisolone and the ganglioside GM1 on acute spinal cord injury in rats

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Recent clinical trials have reported that methylprednisolone sodium succinate (MP) or the monosialoganglioside GM1 improves neurological recovery in human spinal cord injury. Because GM1 may have additive or synergistic effects when used with MP, the authors compared MP, GM1, and MP + GM1 treatments in a graded rat spinal cord contusion model. Spinal cord injury was caused by dropping a rod weighing 10 gm from a height of 1.25, 2.5, or 5.0 cm onto the rat spinal cord at T-10, which had been exposed via laminectomy. The lesion volumes were quantified from spinal cord Na and K shifts at 24 hours after injury and the results were verified histologically in separate experiments. A single dose of MP (30 mg/kg), given 5 minutes after injury, reduced 24-hour spinal cord lesion volumes by 56% (p = 0.0052), 28% (p = 0.0065), and 13% (p > 0.05) in the three injury-severity groups, respectively, compared to similarly injured control groups treated with vehicle only. Methylprednisolone also prevented injury-induced hypothermia and increased body weight loss in the spine-injured rats. When used alone, GM1 (10 to 30 mg/kg) had little or no effect on any measured variable compared to vehicle controls; when given concomitantly with MP, GM1 blocked the neuroprotective effects of MP. At a dose of 3 mg/kg, GM1 partially prevented MP-induced reductions in lesion volumes, while 10 to 30 mg/kg of GM1 completely blocked these effects of MP. The effects of MP on injury-induced hypothermia and body weight loss were also blocked by GM1. Thus, GM1 antagonized both central and peripheral effects of MP in spine-injured rats.

Until this interaction is clarified, the authors recommend that MP and GM1 not be used concomitantly to treat acute human spinal cord injury. Because GM1 modulates protein kinase activity, protein kinases inhibit lipocortins, and lipocortins mediate anti-inflammatory effects of glucocorticoids, it is proposed that the neuroprotective effects of MP are partially due to anti-inflammatory effects and that GM1 antagonizes the effects of MP by inhibiting lipocortin. Possible beneficial effects of GM1 reported in central nervous system injury may be related to the effects on neural recovery rather than acute injury processes.

KEY WORDS • methylprednisolone • ganglioside • potassium • sodium • spinal cord injury • rat

Two recent clinical trials have tested treatments that improve neurological recovery in human spinal cord injury. In the first trial, the second National Acute Spinal Cord Injury Study (NASCIS 2) showed that methylprednisolone sodium succinate (MP) improves neurological recovery when given in high doses and within 8 hours after injury.14,15,131 Patients receiving MP more than 8 hours after injury may have a worse recovery than placebo-treated patients.15 Because the doses of MP required for neuroprotection greatly exceed that needed to activate glucocorticoid receptors and are closer to doses that prevent lipid peroxidation, Hall and colleagues55,63 proposed that MP acts primarily as a free radical scavenger rather than as a glucocorticoid hormone. In the second trial, Geisler, et al.45,46 reported that the monosialoganglioside GM1 improved motor recovery when started 48 to 72 hours after spinal cord injury and after treatment with a low-dose course of MP. In animal studies, GM1 facilitated neurite growth,21,50,100,103 attenuated retrograde degeneration,104,105,107,123 and improved recovery after lesions of the basal ganglia;112 GM1 also prevented glutamate-mediated excitotoxicity,39 reduced ischemic cerebral edema,84 and modulated protein kinase activity in injured brain tissue.22,48,64,82,83

Methylprednisolone and GM1 may have additive or synergistic effects in spinal cord injury. However, the effects of combined MP and GM1 treatments have not been studied before in acute spinal cord injury models. We therefore compared the effects of MP, GM1, and MP + GM1 on spinal cord lesion volumes and the histological appearance of the spinal cords at 24 hours.
Definitions of Abbreviations

ANCOVA = analysis of covariance
ANOVA = analysis of variance
C_a = maximum depth of cord compression
C_i = maximum time of cord compression
CVF = cell volume fraction
D1 = 1st segment of distal cord (adjacent to impact site)
D2 = 2nd segment of distal cord (4 mm from impact site)
G = average gradient of Na or K across cellular membranes
GM1 = monosialoganglioside
[H2O]_a = spinal cord water concentration (ml/gm W)
Imp = impact site
[K_b] = blood K concentration (mM)
[K_e] = extracellular K concentration (mM)
[K_i] = intracellular K concentration (mM)
[K_p] = plasma K concentration (mM)
[K] = tissue K concentration normalized to plasma [Na]_a + [K_b] (mM)
[K_a] = tissue K concentration (µmol/gm wet tissue weight)
MP = methylprednisolone sodium succinate
[Na_b] = blood Na concentration (mM)
[Na_e] = extracellular Na concentration (mM)
[Na_i] = intracellular Na concentration (mM)
[Na_p] = plasma Na concentration (mM)
[Na] = tissue Na concentration normalized to plasma [Na]_a + [K_b] (mM)
[Na_a] = tissue Na concentration (µmol/gm wet tissue weight)
P1 = 1st segment of proximal cord (adjacent to impact site)
P2 = 2nd segment of proximal cord (4 mm from impact site)
SD = standard deviation
SEM = standard error of mean
ΔV = change in cell volume (µl)
V_e = extracellular volume
V_i = intracellular volume
V_t = tissue volume
ΔV/V_t = change in cell volume fraction
W = tissue sample wet weight

Experimental Procedures

All animal protocols were reviewed and approved by the New York University Medical Center Institutional Animal Care and Use Committee. We used 190 adult male Long-Evans hooded rats, each weighing from 450 to 550 gm. The rats were anesthetized with pentobarbital (40 mg/kg intraperitoneally). Rectal temperatures were maintained at 37° ± 0.5°C with a heating pad during surgery and a constant-temperature chamber after surgery. Blood pressure, arterial blood gases levels, and respiratory rates did not differ significantly among the groups.

The spinal cord was exposed via laminectomy at T-10 and a 10-gm rod was dropped from a height of 1.25 (12.5 gm-cm), 2.50 (25.0 gm-cm), or 5 (50.0 gm-cm) cm directly onto the cord. The contusion device monitored rod and vertebral column movement with ± 8-μm and ± 0.01-msec precision by means of digital optical potentiometers. Impact velocities were estimated from the rod trajectory during the 2-msec period before impact. Relative movements of the rod and vertebral column give the maximum depth (C_a) and time (C_t) of cord compression. The ratio C_a/C_t represents the mean compression rate of the spinal cord and is the best predictor of 24-hour lesion volumes in contused spinal cords.

Methylprednisolone sodium succinate powder was dissolved in 0.5 to 1.0 ml normal saline and given intravenously 5 minutes after spinal cord injury; GM1 was likewise prepared in saline and injected 5 minutes or 3 hours after injury.* In rats receiving both MP and GM1, we prepared and injected the drugs in separate syringes but with the same total fluid volume. We chose to treat the rats shortly after injury in order to maximize any neuroprotective effects that MP and GM1 might have. Each treatment protocol was evaluated after the three weight-drop contusions described above. All treatments were coded until data analyses were completed.

Table 1 summarizes the experimental and control groups. For the tonic study, eight unoperated rats served as normal controls, four rats underwent a laminectomy only (sham), and four rats received a laminectomy and a single 30-mg/kg bolus of MP (sham-MP). The experimental rats were randomly assigned to one of seven treatment protocols (21 or 22 in each group). Each of the seven treatment groups had three subgroups consisting of seven or eight rats undergoing a weight-drop contusion of 12.5, 25.0, or 50.0 gm-cm. These groups received one of the following: vehicle (saline); a single bolus dose of 30 mg/kg of MP (MP-1) administered at 5 minutes after injury; 10 mg/kg of GM1 (G10) or 30 mg/kg of GM1 (G30) administered at 5 minutes and at 3 hours after injury; or a combination of MP-1 and GM1 doses of 3 mg/kg (MG3), 10 mg/kg (MG10), or

* Methylprednisolone sodium succinate manufactured by Upjohn Co., Kalamazoo, Michigan; GM1 supplied by Fidia Research Laboratories, Abano Terme, Italy.

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30 mg/kg (MG30) administered at 5 minutes and at 3 hours after spinal cord injury. In addition, three groups of eight or nine rats were injured with 25.0-gm-cm contusions and treated with vehicle, MP-1, or MG30 for histological assessment at 24 hours after injury.

Tissue Ionic Analyses

At 24 hours after injury, the rats were anesthetized with pentobarbital (40 mg/kg intraperitoneally) and decapitated. Six cord samples were obtained for ionic studies: one sample from the site of impact, two from proximal cord (P1 and P2), two from neighboring distal cord (D1 and D2), and one from the T-1 spinal cord level. Each sample was approximately 4 mm in length. The samples were weighed (± 0.1 mg) to obtain wet weight, then dried overnight in a vacuum chamber at 100°C and reweighed to obtain dry weight; they were then dissolved in concentrated nitric acid, ashed on a hotplate, diluted with deionized water, and analyzed for Na and K by air-acetylene flame atomic absorption spectroscopy.14,13,13,136

Blood samples were also obtained from each rat to determine hematocrit, blood Na and K concentrations ([Na]b and [K]b), and plasma Na and K concentrations ([Na]p and [K]p). Following decapitation, blood was collected into test tubes coated with Na-free heparin to prevent clotting.† A portion of heparinized blood was placed into capillary tubes and centrifuged until the blood cells were well packed; the hematocrit was then measured visually on a scale. An aliquot of whole blood was analyzed by atomic absorption analysis for Na and K. The remainder was centrifuged and clear plasma supernatant was collected for atomic absorption analysis.

We divided tissue Na and K contents by wet weight to obtain concentrations, expressed as units of μmol/gm of wet tissue ([Na]t and [K]t). Tissue water concentrations were calculated from the formula: (wet weight – dry weight)/wet weight. Since wet weight – dry weight represents the weight of water in the tissue and 1 ml of water weighs 1 gm, water concentrations are given in ml/gm of wet tissue so that ionic and water concentration units are consistent. Units of blood ([Na]b and [K]b) and plasma ([Na]p and [K]p) concentration are expressed as μmol/gm of blood and μmol/ml (or mM) of plasma, respectively.

To correct for bound or sequestered ions in the tissue, we normalized spinal cord [Na]t and [K]t to plasma levels by multiplying with ([Na]b + [K]b)/([Na]t + [K]t) to obtain [Na]n and [K]n, in units of mM. This correction assumes that tissue fluids are isotonic with plasma. In most cases, values of [Na]t and [K]t were 5% to 6% lower than [Na]b and [K]b, suggesting that a fraction of [Na]t and [K]t, may be bound or sequestered. We used [Na]n and [K]n, as well as [Na]p and [K]p, to assess spinal cord damage. Because [Na]t and [K]t more accurately represent soluble tissue Na and K concentrations, we used lesion volumes calculated from [Na]n and [K]n.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>No. of Rats</th>
<th>Summary of Treatment</th>
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<tbody>
<tr>
<td>control</td>
<td>normal 8</td>
<td>no surgery or SCI; no treatment</td>
</tr>
<tr>
<td>sham</td>
<td>4</td>
<td>laminectomy; 1 ml normal saline at 5 min</td>
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<tr>
<td>sham-MP</td>
<td>4</td>
<td>laminectomy; 30 mg/kg of MP at 5 min</td>
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<tr>
<td>experimental</td>
<td>vehicle 21</td>
<td>SCI; saline at 5 min, 6 hrs, 12 hrs, and 18 hrs</td>
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<tr>
<td></td>
<td>MP-1 22</td>
<td>SCI; 50 mg/kg of MP at 5 min; normal saline at 3 hrs</td>
</tr>
<tr>
<td></td>
<td>G10 22</td>
<td>SCI; 10 mg/kg of GM1 at 5 min and at 3 hrs</td>
</tr>
<tr>
<td></td>
<td>G30 21</td>
<td>SCI; 30 mg/kg of GM1 at 5 min and at 3 hrs</td>
</tr>
<tr>
<td></td>
<td>MG3 21</td>
<td>SCI; 30 mg/kg of MP + 3 mg/kg of GM1 at 5 min; 3 mg/kg of GM1 at 3 hrs</td>
</tr>
<tr>
<td></td>
<td>MG10 21</td>
<td>SCI; 30 mg/kg of MP + 10 mg/kg of GM1 at 5 min; 10 mg/kg of GM1 at 3 hrs</td>
</tr>
<tr>
<td></td>
<td>MG30 21</td>
<td>SCI; 30 mg/kg of MP + 30 mg/kg of GM1 at 5 min; 30 mg/kg of GM1 at 3 hrs</td>
</tr>
</tbody>
</table>

* Treatment groups are listed along with the number of surviving rats from which ionic and histological data were obtained. The times listed refer to the time after spinal cord contusion when treatment was given. MP = methylprednisolone; SCI = spinal cord injury.

Cell Volume Fraction Determinations

Fluids in different tissue compartments are isotonic because small differences in osmolality across membranes can produce high pressures. For example, according to the Van Hoff's equation, 1 mM of ionic osmolality difference will generate 19.7 mm Hg of pressure. Because Na, K, and associated anions exert greater than 95% of tissue fluid osmolarity, sums of Na and K concentrations should be approximately equal in intracellular ([Na]i and [K]i) and extracellular ([Na]o and [K]o) fluids. Likewise, since macromolecules in plasma compensate for blood pressure differences, sums of Na and K concentrations should be similar in plasma and extracellular fluids. Therefore, isotonicity can be expressed by the following equation:

\[ [Na]_i + [K]_i = [Na]_o + [K]_o = [Na]_p + [K]_p \]  

1

If so, transmembrane Na and K gradient (G) values also should be equal; that is,

\[ G = [Na]_p - [Na]_i = [K]_o - [K]_i \]  

2

By definition, tissue ionic contents equal sums of intracellular and extracellular ionic contents,

\[ [Na]_i V_i = [Na]_o V_o + [Na]_p V_p \]  

3

and

\[ [K]_i V_i = [K]_o V_o + [K]_p V_p \]  

4

† Na-free heparin supplied by Sigma Chemical Co., St. Louis, Missouri.

where \( V_t \), \( V_o \), and \( V_e \) are tissue, intracellular and extracellular volumes, respectively. Subtracting Equation 4 from Equation 3 yields:

\[
([Na]\_a - [K])V_i = ([Na]\_a - [K])V_e + ([Na]\_a - [K])V_t. \tag{5}
\]

Since \([Na]\_a = G + [Na]\_a\) and \([K] = [K]_a - G\), substitution into Equation 5 gives:

\[
([Na]\_a - [K])V_i = ([Na]\_a - [K])V_e + ([Na]\_a - [K])V_t + 2G. \tag{6}
\]

Rearranging terms gives:

\[
([Na]\_a - [K]) = ([Na]\_a - [K])V_t + 2G. \tag{7}
\]

Since \( V_t = V_i + V_e \), the equation simplifies to:

\[
([Na]\_a - [K] = ([Na]\_a - [K])_t + 2G. \tag{8}
\]

Equation 8 states that \([Na]\_a - [K]\) is linearly related to \( V_t/V_i \) with a slope of twice the gradient and a y intercept of \([Na]\_a - [K]\). The ratio of cell to tissue volume \( V_t/V_i \) is equivalent to the cell volume fraction (CVF) of the tissue. To calculate \( V_t/V_i \), from \([Na]\_a - [K] \), we assumed that \([Na]\_a - [K] = [Na]\_a - [K]_a\) and that \( G = -120\) mV. Ion-selective microelectrode recordings have shown that \([Na]\_a\) and \([K]\) approach \([Na]\_a\) and \([K]\) within 30 minutes of injury. \(^2\) Calculated \( V_t/V_i \) values are expressed as percentages.

**Lesion Volume Assessment**

The change in \( V_t/V_i \) \((\Delta V/V_i)\) reflects the volume of cells that have lost theirionic gradients as a result of injury. To estimate \( \Delta V/V_i \), we subtracted a normal CVF value of 0.70 from the calculated \( V_t/V_i \) of each cord sample. Since 1 \( \mu l \) of tissue weighs about 1 mg, multiplying \( \Delta V/V_i \) by the tissue wet weight in milligrams gives the cell volume lost \( (\Delta V) \) in microliters. The sum of the \( \Delta V \) in P2, P1, site of impact, D1, and D2 tissue samples represents microliters of cells lost within 1 cm of the impact center. We refer to summed \( \Delta V \) values as the ionic lesion volume.

Ionic lesion volumes thus depend on three variables: \([Na]\_a - [K]_a\), \([Na]\_a), and wet weight. The calculations assume that \([Na]\_a - [K] = [Na]\_a - [K]_a\) and that \( G = -120\) mV. Concentrations of \([Na]\_a\) and \([K]\_a\) closely approximate \([Na]\_a - [K]\) at 24 hours after injury. Small inaccuracies of the assumed gradient value will not invalidate treatment effects as long as the gradient values are similar in all the treatment groups. To rule out general effects of treatment or injury on transmembrane ionic gradients, we estimated gradient values in blood samples by linear regression of \([Na]\_a - [K]\) and hematocrit. Since hematocrit is essentially the CVF of blood, \([Na]\_a - [K]\) should correlate linearly with hematocrit, and the slope of the relationship should equal twice the gradient value while the y intercept should be close to \([Na]\_a - [K]_a\).

**Histological Assessment**

Separate histological studies were performed in 25 rats injured with 25.0-gm-cm contusions and treated with vehicle (eight rats), MP-1 (eight rats), or MG30 (nine rats) (Table 1). The rats were anesthetized with pentobarbital and perfused intra-aortically with 10% formalin at 24 hours after injury. After immersion fixation in 10% formalin for 1 week, the spinal cords were embedded in paraffin, serially sectioned on the horizontal plane, and stained with hematoxylin and eosin. Representative horizontal sections passing through the central canal were selected for analysis.

To assess the histological appearance of the lesion site, each of the authors ranked the sections subjectively from worst to best, based on the extent of hemorrhage, necrosis, and white-matter preservation at the lesion center. To compare specific histological characteristics, each spinal cord section was scored for hemorrhage (0 = none, 1 = petechial, 2 = patchy, 3 = confluent, and 4 = gross), necrosis (0 = none, 1 = scattered, 2 = patchy, 3 = confluent, and 4 = gross), and white-matter preservation at the impact site (0 = intact, 1 = bilateral thick, 2 = unilateral thick, 3 = thin, and 4 = no rim). The term "confluent" denotes large areas of hemorrhage or necrosis that occupy less than 50% of the impact site, while the term "gross" indicates involvement of more than 50%. The scores were summed for a total possible histological score of 0 to 12, with higher numbers indicating more severe lesions.

**Statistical Analyses**

We used several statistical approaches to assess changes in outcome measures. First, we used paired t-tests to compare pre- and postinjury values of blood pressure and body weight within injury-treatment groups. Second, to ascertain whether treatments altered lesion volumes across injury groups, we compared individual groups treated with MP-1 and vehicle control groups, using analysis of covariance (ANCOVA) with \( C_2/C_3 \) as the covariate and multiplying p values by 6 to compensate for multiple comparisons. Third, to compare treatment effects within injury levels, we used analysis of variance (ANOVA) and the Bonferroni-Dunn post hoc test to identify groups that differed significantly from MP-1 or vehicle control groups. Fourth, to assess injury or treatment effects on systemic variables, we pooled data for each injury level or treatment group. We then applied ANOVA and the Bonferroni-Dunn post hoc test to identify significant differences between individual groups and MP-1 or vehicle control groups. The ANOVA and ANCOVA tests were carried out using a statistical program\(^\ddagger\) for Macintosh computers.

To assess treatment effects on histological changes, we evaluated the mean ranks of the groups by ANOVA, using Scheffe's post hoc test to compare the ranks of individual groups. We also used ANOVA and Scheffe's post hoc test to compare summed and individual histological scores among the treatment groups. Finally, we used the nonparametric Mann-Whitney U-test to confirm the significance of individual histological

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\(^\ddagger\) SuperANOVA statistical program supplied by Abacus Concepts, Inc., Berkeley, California.
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![Graphs showing compression rate versus impact velocity and lesion volume versus compression rate.](image)

Fig. 1. Scatterplots of compression rate versus impact velocity (left) and lesion volume versus compression rate (right). Left: The regression line for all groups is shown. Points representing individual injury-treatment groups are closely grouped. Right: Regression lines are shown for vehicle control (VG) (slope = 36.0 ± 11.4, y intercept = 12.8 ± 5.5, r = 0.5872, t = 3.16) and MP-1 (slope = 62.5 ± 12.1, y intercept = -8.7 ± 5.8, r = 0.7639, t = 5.16) groups.

score differences, multiplying p values by 3 to compensate for multiple comparisons.

All measured data in the text and tables are expressed as mean values ± SD, with two exceptions: 1) variances of linear regression slopes and y intercepts are given in standard error of the means (SEM's); and 2) variances of lesion volumes in the figures are expressed in SEM's. The criterion for statistical significance was a p value less than 0.05.

**Results**

*Tissue and Blood Values in Normal or Sham-Injured Controls*

In eight normal unoperated rats, concentrations of spinal cord water (H₂O), Na⁺ ([Na⁺]w) and K⁺ ([K⁺]w) were 0.672 ± 0.005 ml/gm wet weight, 61.4 ± 3.2 μmol/gm, and 97.6 ± 1.5 μmol/gm, respectively. The normal thoracic spinal cord Na⁺ - [K⁺] value was -33.2 ± 1.4 mM, [Na⁺]w - [K⁺] was 134.4 ± 1.3 mM, and Vᵢ/Vᵣ was 69.8% ± 0.9%, assuming that G = -120 mM. The mean normal blood [Na⁺]w - [K⁺] value was 26.6 ± 2.2 mM and Vᵢ/Vᵣ was 44.9% ± 0.8%, assuming that G = -120 mM. Calculated blood Vᵢ/Vᵣ values did not differ significantly from measured hematocrit values of 0.463 ± 0.009 (p > 0.05, two-tailed t-test).

Methylprednisolone did not significantly alter blood or spinal cord [Na⁺]w + [K⁺], [Na⁺] - [K⁺], Vᵢ/Vᵣ, or hematocrit values in sham-injured rats (see Table 5). Because MP-treated and untreated sham-operated animals did not differ significantly from each other in any of the measured variables, we pooled the two sham-operated groups. Calculated Vᵢ/Vᵣ values in the normal, vehicle-treated sham injury, and MP-treated sham injury groups were all close to 0.70 and did not differ significantly from each other (p > 0.05, ANOVA).

**Contusion Parameters**

Spinal cord contusion parameters were very consistent across treatment groups. Figure 1 left shows a scatterplot of impact velocity and spinal cord compression rate (Cᵢ/Cᵣ). Table 2 lists the mean impact velocities in the 12.5-, 25.0-, and 50.0-gm-cm injury groups. The impact velocities were typically 5% to 7% smaller than

| TABLE 2
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<tbody>
<tr>
<td><strong>Contusion parameters in spine-injured rats</strong></td>
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<tr>
<td>Weight Drop (gm-cm)</td>
<td>No. of Rats</td>
<td>Ideal Velocity (m/sec)†</td>
<td>Impact Velocity (m/sec)</td>
<td>Deviation From Ideal (m/sec)‡</td>
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<tr>
<td>12.5</td>
<td>49</td>
<td>0.495</td>
<td>0.458 ± 0.012 (2.6%)</td>
<td>-0.037</td>
</tr>
<tr>
<td>25.0</td>
<td>50</td>
<td>0.700</td>
<td>0.665 ± 0.026 (3.9%)</td>
<td>-0.035</td>
</tr>
<tr>
<td>50.0</td>
<td>49</td>
<td>0.990</td>
<td>0.954 ± 0.028 (2.9%)</td>
<td>-0.036</td>
</tr>
</tbody>
</table>

*Means are expressed ± standard deviation (SD of contusion parameters (with SD as a percentage of the means in parentheses) in all rats subjected to spinal cord injury and analyzed for spinal cord ion changes.
†The theoretical velocity of a weight dropped in a friction-free environment, calculated from Galileo's law.
‡Deviations of mean impact velocities from ideal values calculated from the 2-msec trajectory just before impact.
§Rates obtained by dividing the maximum cord compression depth by the compression time. Compression rates were generally more variable than impact velocities.

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ideal free-fall values in all groups and had SD's that were consistently less than 5% of the group means. Compression rates were more variable with SD's that were 7% to 15% of the group means. Neither impact velocity nor cord compression rates differed significantly among treatment groups (p > 0.05, ANOVA). Impact velocities linearly predicted spinal cord compression rates (C₀/C₀) with a correlation coefficient of 0.992, a slope of 0.585 ± 0.017 (mean ± SEM), and a y intercept of 0.071 ± 0.012 (mean ± SEM).

Injury significantly increased [H₂O]₀ and [Na₉]₀, but significantly reduced [K]₀ at the impact site of vehicle-treated rats. The lesions are described at 12.5 g-cm in normal, sham-injury, and vehicle-injury groups. The value of [Na₉]₀ at the impact site increased from -33.0 ± 3.9 mM in normal rats to -30.5 ± 5.9, 23.1 ± 8.0, 37.9 ± 7.1, and 51.8 ± 7.8 mM at 24 hours after sham, 12.5-, 25.0-, and 50.0-gm-cm contusions, respectively. Calculated impact site V₀/V₀ values fell from 69.7% ± 2.0% in unoperated normal rats to 69.6% ± 4.0%, 42.6% ± 4.5%, 35.7% ± 3.1%, and 31.6% ± 4.5% after sham, 12.5-, 25.0-, and 50.0-gm-cm contusions, respectively.

Spinal cord compression rates correlated linearly with spinal cord lesion volumes (Fig. 1 right). In vehicle-treated rats, including vehicle-treated sham injuries (four rats), linear regression analysis revealed a slope of 55.9 ± 6.6 μl (± SEM) of cells per m/sec increase in compression rate, a y intercept of 2.9 ± 2.9 (± SEM), and a correlation coefficient of 0.8700 (t = 8.46, p < 0.0001). When data from all treatment and injury groups were pooled, cord compression rates correlated linearly with lesion volumes, yielding a slope of 70.1 ± 9.6, a y intercept of -4.2 ± 4.7, and a correlation coefficient of 0.8584 (t = 7.3, p < 0.0005).

**Effect of Treatment on Lesion Volumes**

Methylprednisolone significantly reduced ionic lesion volumes (Figs. 1 right and 2) compared to vehicle controls. In the vehicle control group, the mean values (± SEM) of lesion volumes were 22.2 ± 2.7, 32.3 ± 1.0, and 34.2 ± 2.3 μl at 24 hours after 12.5-, 25.0-, and 50.0-gm-cm contusions, respectively. In the MP-1 group, lesion volumes were reduced by 56% (p = 0.0052), 28% (p = 0.0065), and 13% (p > 0.05) after 12.5-, 25.0-, and 50.0-gm-cm contusions, respectively (ANOVA). The ANCOVA values revealed very significant treatment effects; that is, MP-1 lesion volumes were significantly smaller than those in the vehicle control (p = 0.0084), G10 (p = 0.0006), G30 (p = 0.0012), MG3 (p = 0.042), MG10 (p = 0.003), and MG30 (p = 0.003) groups.

Treatment with GM1 alone showed no significant effect on lesion volumes. Rats treated with 10 mg/kg or 30 mg/kg of GM1 (G10 and G30), given at 5 minutes and at 3 hours after injury, did not differ significantly from vehicle controls at each injury level (p > 0.10, ANOVA). Correcting for contusion differences between groups (ANCOVA) did not change this conclusion. For example, lesion volumes in the G10 and G30 groups did not differ significantly from those in the vehicle control group (p > 0.50, ANCOVA).

The neuroprotective effects of MP were antagonized by the administration of GM1. In rats injured with 12.5-gm-cm contusions, ANOVA indicated significantly greater lesion volumes in the MG10 (p = 0.0029) and MG30 (p = 0.0041) groups compared to the MP-1 group. Among rats injured with 50-gm-cm contusions, the MG3 (p = 0.0019), MG10 (p = 0.0019), and MG30 groups showed significantly greater lesion volumes compared to the MP-1 group (p = 0.0019, MG10 (p = 0.0019), and MG30 groups showed significantly greater lesion volumes compared to the MP-1 group.

*Values are means ± standard deviations of plasma Na and K concentrations ([Na₉]₀ + [K]₀), spinal cord wet weight concentrations ([Na₉]₀ + [K]₀), tissue concentrations ([Na₉]₀ + [K]₀), and cell volume fractions (intracellular/tissue volume, V/V₀) at the impact site of vehicle-treated rats compared with other control groups. MP = methylprednisolone.*
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### TABLE 4

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Rats</th>
<th>Mean Rank$$</th>
<th>Mean Histological Scores</th>
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<tr>
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<td>Hemorrhage</td>
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<td>Vehicle</td>
<td>8</td>
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<td>2.13 ± 0.64</td>
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<td>8</td>
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<td>1.25 ± 0.46†</td>
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<tr>
<td>MG30</td>
<td>9</td>
<td>6.56 ± 5.05‡</td>
<td>3.00 ± 0.71‡</td>
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* Mean ranks and histological scores (± standard deviation) were compared by ANOVA using Scheffe’s post hoc test. Significant differences from vehicle-treated rats: † = p < 0.05; significant differences from a bolus dose of methylprednisolone (MP-1); ‡ = p < 0.05. For lesion scoring system, see Materials and Methods: Histological Assessment (higher numbers denote more severe lesions). See Table 1 for summary of treatment.

§ Ranks were obtained by arranging selected slides in order from worst to best (1 to 25).

(\(p = 0.0092\)) groups had significantly larger lesion volumes compared to the MP-1 group. The MG10 and MG30 groups had greater lesion volumes than the MP-1 group across injury groups \((p \leq 0.003,\) ANCOVA) but did not differ from vehicle controls \((p > 0.1,\) ANCOVA).

**Effect of Treatment on Histological Appearance of the Impact Site**

In the MP-1 group, the histological appearance of the spinal cords was improved compared to those with vehicle and MG30 treatments; the MP-1-treated rats had a significantly better histological rank than the MG30-treated rats \((p = 0.0005,\) ANOVA Scheffe’s test) but did not have a significantly better mean histological rank compared to vehicle-treated rats \((p = 0.1017).\) The mean rank of the MG30 group was significantly worse than the vehicle control group \((p = 0.039).\) Table 4 lists the histological ranks.

The MP-1-treated rats had less hemorrhage than the vehicle-treated rats \((p = 0.0324,\) ANOVA Scheffe’s test), but their necrosis and white matter scores did not differ significantly. As shown in Table 4, MG30-treated animals had the worst lesions, with most cords showing gross hemorrhage \((p = 0.0001\) compared with MP-1-treated rats and \(p = 0.273\) compared with vehicle-treated animals, ANOVA Scheffe’s test), necrosis involving more than 50% of the impact site, and thin rims of preserved white matter. Nonparametric tests of the histological scores yielded similar conclusions.

**Effect of Injury and Treatment on Body Weight Loss and Plasma Osmolarity**

Table 5 lists preoperative body weights and percentage weight changes in groups pooled by injury or treatment. Operated rats lost body weight compared to unoperated normal rats; sham-injured rats lost a mean of 8.3% ± 1.3% of body weight and increasing injury severity did not increase weight loss. Thus, the weight losses are due to the operation and not to spinal cord injury *per se*. Spinal cord injury reduced body weight loss since vehicle-treated spine-injured rats lost only 3.8% ± 2.3% of their body weight.

Treatment with MP increased body weight loss in spine-injured rats. As shown in Table 5, all MP-treated rats lost 7% to 8% of body weight, irrespective of GM1 treatment or injury severity, significantly more than vehicle control, G10, or G30 groups \((p < 0.05,\) ANOVA Scheffe’s test). The MP-1, MG3, MG10, and MG30 groups lost 7.3% ± 2.8%, 7.6% ± 1.5%, 7.6% ± 3.9%, and 7.2% ± 2.4% of body weight, respectively. However, rats receiving MP and G10 or G30 lost 4.8% ± 3.0% and 5.4% ± 1.6%. Thus, GM1 partially blocked the effects of MP on body weight loss in injured rats.

Spinal cord injury significantly reduced \([Na]^+ + [K]^+)\. In normal and sham-injured rats, \([Na]^+ + [K]^+)\ values were 144.6 ± 2.5 mM and 145.6 ± 4.8 mM, respectively, not significantly different from each other \((p > 0.05,\) ANOVA). Thus, anesthesia and laminectomy did not alter \([Na]^+ + [K]^+)\ values. In vehicle-treated spine-injured rats, \([Na]^+ + [K]^+)\ fell to 136.8 ± 5.1 mM, lower than normal or sham-injured rats \((p < 0.005,\) ANOVA). Since \([K]^+)\ values were between 4 and 6 mM in all rats, the fall in \([Na]^+ + [K]^+)\ signifies hyponatremia.

Treatment with MP prevented spinal cord injury-induced hyponatremia. In the MP-1 group, \([Na]^+ + [K]^+)\ was 149.4 ± 4.3 mM, significantly higher than 136.8 ± 5.1 mM in the vehicle-treated spine-injured rats \((p < 0.005,\) ANOVA Bonferroni-Dunn test). Although GM1 treatment alone had little effect on injury-induced hyponatremia, it blocked the effects of MP on injury-induced hyponatremia. In rats given 3 mg/kg of GM1 in addition to MP, \([Na]^+ + [K]^+)\ was 141.2 ± 3.3 mM, significantly lower than in MP-1-treated rats \((p < 0.01,\) ANOVA Bonferroni-Dunn test). In rats receiving 10 mg/kg or 30 mg/kg of GM1 in addition to MP, \([Na]^+ + [K]^+)\ was 142.5 ± 3.4 and 140.0 ± 2.7 mM, both significantly lower than in MP-1-treated rats \((p < 0.01,\) ANOVA Bonferroni-Dunn test).

**Effect of Injury and Treatment on Blood Hematocrit and V/V, Values**

Normal and sham-injured rats had hematocrits of 46.3% ± 2.6% and 45.3% ± 2.7%, respectively, not significantly different from each other \((p > 0.05,\) ANOVA). Thus, although surgery and blood loss during laminectomy did not reduce the hematocrit, spinal cord injury did. As shown in Table 5, the hematocrit fell significantly \((p < 0.005,\) ANOVA Bonferroni-
TABLE 5

<table>
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<tr>
<th>Treatment Group</th>
<th>No. of Rats</th>
<th>Weight (gm)</th>
<th>Body Weight Change (%)</th>
<th>Hematocrit (%)</th>
<th>Blood V/Vm (mM)</th>
<th>[Na]p + [K]p (mM)</th>
<th>T-1 V/Vm (mM)</th>
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<td>69.8 ± 2.3</td>
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<td>455 ± 4</td>
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<td>12.5</td>
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<td>485 ± 28</td>
<td>-7.1 ± 3.0†</td>
<td>36.0 ± 4.5†$</td>
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<td>140.9 ± 5.9</td>
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<td>25.0</td>
<td>50</td>
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<td>34.2 ± 4.1†‡</td>
<td>140.0 ± 2.7†‡</td>
<td>66.3 ± 3.6‡</td>
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</table>

*Values are means ± standard deviations of preoperative body weights, percentage change of body weight, hematocrit, blood cell volume fraction (intracellular/tissue volume, V/Vm), plasma Na and K concentrations ([Na]p + [K]p), and calculated V/Vm of cord samples obtained at T-1 (T-V/Vm). For explanation of treatment, see Table 1. MP = methylprednisolone. Significant differences (ANOVA, Bonferroni-Dunn post hoc test) from normal control values: † = p < 0.005; from vehicle controls: ‡ = p < 0.005; and from sham controls: $ = p < 0.005.

Dunn test) in all injury groups, regardless of treatment. Methylprednisolone and GM1 treatment did not significantly alter the extent of injury-induced hematocrit changes (p > 0.05, ANOVA). Changes in hematocrit also did not correlate with injury severity. Thus, the reduction in hematocrit appears to be related to spinal cord injury, but occurred regardless of spinal cord injury severity or treatment with MP or GM1.

Blood V/Vm values accurately predicted measured hematocrit. We calculated V/Vm from blood [Na] − [K], [Na]p − [K]p, and Equation 8, assuming that G = -120 mM. Calculated values of V/Vm were slightly lower than hematocrit but not significantly so. As shown in Table 5, mean V/Vm values did not differ markedly from the measured hematocrit in any treatment or injury group (p > 0.10, ANOVA); blood V/Vm values were within 8% of the measured hematocrit in all groups and within 2% in most. The precision of our hematocrit measurements is approximately 2% and lysis of blood cells may have occurred in some samples during hematocrit measurements. Linear regression analysis of blood V/Vm and hematocrit revealed a correlation coefficient of 0.9275 (t = 12.2, p < 0.0001), a slope of 1.00 ± 0.08 (± SEM), and a y intercept of -0.01 ± 0.03 (± SEM).

Effect of Injury and Treatment on Blood Gradients

The lesion volume calculations assume that neither treatment nor injury changed the value of the gradient. To test this assumption, we estimated the values of gradient in blood samples by linear regression of [Na] − [K] and hematocrit. According to Equation 8, the slope of the relationship should be equal to twice the gradient and the y intercept should be equal to [Na]p − [K]p. If treatment has a general effect on transmembrane ionic gradients, it should be apparent.

Linear regression analysis of blood [Na] − [K] versus hematocrit from all experiments revealed a slope of -231.2 ± 5.5 mM (± SEM) and a y intercept of 132.8 ± 2.0 mM (± SEM) with a correlation coefficient of -0.959. The slope should equal twice the gradient and the y intercept should equal [Na]p − [K]p. The slope therefore suggests that the value of the gradient is -115.6 ± 5.5 mM (mean ± SEM) and [Na]p − [K]p is 132.8 ± 2.0 mM (mean ± SEM). These values are close to the assumed gradient value of -120 mM and the measured [Na]p − [K]p value of 131 ± 1.6 mM (mean ± SEM).

Values of G in blood samples were estimated by linear regression of [Na] − [K] and hematocrit. These values did not differ significantly among the treatment groups or the injury groups (p > 0.05, ANOVA). As pointed out above, calculated blood V/Vm values accurately predicted hematocrit in all treatment and injury groups despite large differences in [Na]p, hematocrit values among the groups. The values of G in blood therefore appear to be independent of treatment, injury, and changes in [Na]p − [K]p.

Effect of Injury and Treatment of V/Vm at T-1

Injury and treatment altered V/Vm values at the T-1 spinal cord level. The data suggest slight losses in cell volume at T-1 that increased with injury severity (Table 5). Normal and sham-injured rats had mean T-1 V/Vm values of 69.8% ± 2.3% and 67.9% ± 3.1%, respectively. Mean T-1 V/Vm values fell with increasing impact velocity. For example, after 50.0-gm-cm contusions, the mean V/Vm was 65.3% ± 4.3%, significantly less than normal or sham-injured rats (p < 0.005, ANOVA Bonferroni-Dunn test).

Methylprednisolone treatment prevented the injury-induced fall of V/Vm at the T-1 cord level. As shown...
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in Table 5, the T-1 V/V, value was 70.2% ± 3.9% in the MP-1 group compared to 65.4% ± 2.3% in the vehicle-treated group (p < 0.005, ANOVA Bonferroni-Dunn test); this value did not differ significantly from either the sham or normal control group value. Histological examination of the T-1 cord after a 25.0-gm-cm impact revealed gray-matter petechial hemorrhage in the vehicle- and MP+GM1 (MG30)-treated rats but not in rats treated with MP alone.

Discussion

The results indicate that a single 30-mg/kg dose of MP significantly reduced 24-hour lesion volumes in injured rat spinal cords. This neuroprotective effect diminished with injury severity. Although GM1 had remarkably little effect on all outcome measures when given alone, it blocked the neuroprotective effects of MP partially at a 3-mg/kg dose and completely at a 10- or 30-mg/kg dose. Spine-injured rats developed significant hyponatremia and suffered loss of body weight; MP prevented the hyponatremia but increased the weight loss. The GM1 treatment partially antagonized these effects of MP on hyponatremia and weight loss.

This study is the first detailed description of the [Na]e − [K]i approach to assessing lesion volumes in injured spinal cords. It is also the first study comparing the effects of MP and GM1 in a graded spinal cord injury model. In this discussion, we will examine first the [Na]e − [K]i approach to estimating lesion volumes and then the effects of MP on lesion volumes and injury-induced hyponatremia, the lack of effect by GM1 on lesion volumes, the robust blockade of MP effects by GM1, the mechanisms of MP neuroprotection, and GM1 interaction and the implications of these findings.


The relationship of [Na]e − [K]i to V/V, provides a uniquely simple, efficient, and accurate approach to measuring tissue damage. The relationship is derived from a simple robust assumption that the sums of Na and K concentrations are similar in intracellular and extracellular fluids. The method does not require fixation or preservation of the tissue samples. Since neither Na nor K degrade, once the tissue is removed from the body total tissue Na and K contents should not change. Measurements of tissue Na and K concentrations are more accurate and precise than morphometric measurements of lesion areas. Finally, by multiplying calculated V/V, values with tissue wet weight, the volume of cells lost can be expressed in microliters.


In some circumstances, [Na]i − [K]i may deviate from [Na]e − [K]e. For example, shortly after injury, [Na]i is often low and [K]i high. However, [K]i typically returns close to plasma levels within 30 minutes after injury in the rat spinal cord contusion model. Thus, the assumption that [Na]i − [K]i closely approximates [Na]e − [K]i levels is very likely to be true at 24 hours after injury. Care should be taken in interpreting [Na]i − [K]i changes in tissue that may have deranged [Na]e and [K]e values.

Effect of Methylprednisolone on Spinal Cord Injury

The finding that MP reduces the volume of spinal cord lesions at 24 hours confirms extensive studies of spinal cord injury in cats, rats, and humans showing that MP is neuroprotective. Our study, however, is the first to assess the effects of MP in a graded spinal cord injury model. A single 30-mg/kg dose of MP significantly reduced lesion volumes by 56% in mildly contused spinal cords and by 28% in moderately contused cords, but had insignificant effects on severely contused spinal cords.

Spinal cord injury caused significant hyponatremia in the rats. Since the only fluids received by the rats came from intravenous injections, the hyponatremia cannot be due to excessive water intake. The hyponatremia may be due to natriuresis secondary to atrial natriuretic peptide release. A major secretory agent of the heart and central nervous system (CNS) tissues, atrial natriuretic peptide is released by catecholamines that are elevated after spinal cord injury.

Methylprednisolone consistently prevented spine injury-induced hyponatremia and increased the loss of body weight, although the mechanism is unclear. We considered three potential actions of MP: 1) glucocorticoids enhance secretion and activity of atrial natriuretic peptide; 2) high-dose glucocorticoids may have mineralocorticoid side effects promoting renal Na resorption; and 3) glucocorticoids inhibit antiuretic hormone effects. The third action seems most likely since only this mechanism would increase both weight loss and [Na]i + [K]i. This is supported by a rise in hematocrit in MP-treated rats (Table 5).

Effect of GM1 on Spinal Cord Injury

Treatment with GM1 alone had little or no effect on any measured parameters. At 10 or 30 mg/kg, GM1 did not significantly alter weight loss, [Na]i + [K]i, blood hematocrit, or spinal cord tonic shifts compared to vehicle-treated rats. This contrasts with previous studies reporting that monosialic gangliosides significantly reduce ischemic cortical damage and excitotoxic neuronal loss.

Most studies showing beneficial effects of GM1 on neurologically recovery gave the drug daily for weeks after injury. Our results do not rule out beneficial effects of long-term GM1 treatment in spinal cord injury but suggest that any beneficial effects of

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GM1 are not due to the reduction of acute tissue damage. The administration of GM1 may prevent anterograde and retrograde neuronal degeneration and stimulate neural repair mechanisms.\textsuperscript{10,51,103,105}

The effects of MP were antagonized by GM1 in the spine-injured rats. At a dose of 3 mg/kg, GM1 significantly reduced the neuroprotective effects of MP on spinal cord lesion volumes and, at a dose of 10 or 30 mg/kg, it completely blocked these effects. GM1 partly reversed the effects of MP on [Na\textsubscript+] + [K\textsubscript{]} but did not significantly affect MP enhancement of postoperative weight loss. These results suggest that GM1 blocks the mechanisms responsible for neuroprotective effects of MP.

\textit{Mechanisms of Methylprednisolone-Mediated Neuroprotection}

Hall and colleagues\textsuperscript{55,59} have hypothesized that MP protects neurons by scavenging oxygen free radicals. This hypothesis is based on the observation that neuroprotective doses of MP greatly exceed those required for glucocorticoid receptor activation and are close to the doses that inhibit lipid peroxidation in injured spinal cords.\textsuperscript{18,57,58,62} Other antioxidant agents are neuroprotective in spinal cord injury models, including vitamin E\textsuperscript{2,4,5,108} and tircilazad mesylate.\textsuperscript{60,90} The latter is a 21-aminosteroid with no glucocorticoid activity but with neuroprotective effects in both brain and spinal cord injury models.\textsuperscript{17,19,54,56,61} Methylprednisolone, however, is a glucocorticoid with potent anti-inflammatory properties.\textsuperscript{71} Glucocorticoids induce synthesis\textsuperscript{20} and release\textsuperscript{12,41,98} of anti-inflammatory peptides, including lipocortins. Ubiquitously present in the CNS,\textsuperscript{79} lipocortins inhibit calcium-activated phospholipase activity\textsuperscript{1,12,67,98} by binding to membrane phospholipid substrates,\textsuperscript{30,41,98,111} inhibiting membrane-bound protein kinase C which activates phospholipases,\textsuperscript{52,122} or inducing phosphatases which inactivate phospholipases.\textsuperscript{137} Lipocortins inhibit cationic production,\textsuperscript{25-27,98} and prevent the generation of superoxide by phagocytes, perhaps due to inhibition of reduced nicotinamide adenine dinucleotide phosphate oxidase.\textsuperscript{89}

Phospholipase activity increases in injured CNS tissue.\textsuperscript{113,127,128} Phospholipid breakdown releases lipid inflammatory mediators, including prostaglandins and other eicosanoids. These inflammatory substances accumulate in injured spinal cords.\textsuperscript{8,9,31,70,72,78,91,116} Blockade of eicosanoids improves blood flow and reduces edema in injured spinal cords.\textsuperscript{37,42,53,62} Consequently, the anti-inflammatory activity of MP is likely to play a major role in its neuroprotective effects. Hsu and Dimitrijevic\textsuperscript{71} have proposed that the neuroprotective effects of MP are in part due to its anti-inflammatory activity.

\textit{Implications of GM1 Blockade of Methylprednisolone Activity}

The finding that GM1 blocks the effects of MP in spinal cord injury has important clinical implications. Until more is known about the interactions of GM1 and MP, we strongly recommend that the two drugs not be given together to treat acute spinal cord injury in humans. In an early study\textsuperscript{45,46} reporting beneficial effects of GM1 in human spinal cord injury, low-dose MP was given for 48 to 72 hours before GM1 was started. No experimental or clinical data now support the administration of high-dose MP and GM1 together during the first 24 hours after injury.

An antagonistic interaction between GM1 and MP provides insights into the actions of both drugs. The GM1 blockade of glucocorticoid anti-inflammatory mechanisms may explain the neurite growth-enhancing activity of GM1.\textsuperscript{10} Since inflammation promotes neuronal growth\textsuperscript{96,101,102} and glucocorticoids suppress sprouting and regeneration,\textsuperscript{102,103} prolonged glucocorticoid therapy is likely to impair recovery processes. This may explain why patients started on MP ther-
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apy more than 8 hours after spinal cord injury had worse recovery than did the placebo controls in the NASCIS 2.14,15,131

Inflammatory activity may contribute to secondary tissue damage. This may be one reason why high-dose MP reduces tissue damage in injured spinal cords. If so, high doses of glucocorticoids other than MP should also be effective for acute injury. For example, dexamethasone has been used to treat spine-injured patients and earlier animal studies have reported beneficial effects.17,29,30,117,125,126 However, if inflammation plays a key role in subsequent repair and recovery, then delayed treatment with drugs that block glucocorticoid activity maywell enhance recovery.

Our data suggest a dichotomy between acute injury and subacute repair mechanisms. At 6 to 24 hours after injury, a primary concern of therapy is to limit secondary injury processes, mediated in part by inflammation. At later time periods, however, anti-inflammatory therapy may interfere with clean-up and repair mechanisms. Acute injury and recovery phases may well require opposite therapeutic strategies.

The Role of Protein Kinases in Injury

Injury increases intracellular Ca++ activity and cyclic adenosine monophosphate, activating protein kinases that modulate receptors and intracellular and extracellular messengers, such as lipocortin. Glucocorticoids, opioids, growth factors, lipid inflammatory mediators, neurotransmitters, and peptides such as atrial natriuretic factor all interact through protein kinases. Even axons possess receptors to growth factors, inflammatory mediators, and neurotransmitters.69,85 Protein kinases link receptors to the response of cells to injury.

The injury response occurs not only in the injured spinal cord but systemically as well. Spinal cord injury markedly altered plasma osmolality, hematocrit, and body weight. Methylprednisolone significantly reduced these changes. Administration of GM1 alone had little effect on these changes but, when given with MP, significantly reduced the effects of MP on the systemic injury response, suggesting that the interactions between GM1 and MP are not limited to protein kinases in injured tissue.

This study showed that a modulator of protein kinase activity blocks both neuroprotective and systemic effects of MP given shortly after spinal cord injury. If GM1 stimulates protein kinases, then general protein kinase inhibitors such as sphingosine may well enhance neuroprotective effects of MP or may even be directly neuroprotective in acute injury. Thus, the observation that GM1 blocks the neuroprotective effects of MP in spinal cord injury offers interesting and new therapeutic opportunities.

Acknowledgments

We thank Vincent DeCrescito for his invaluable help in carrying out the experiments. Bor-Tom Ng for carrying out the atomic absorption analyses, and Hak Ng for technical assistance with the surgical procedures.

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Manuscript received November 9, 1992.
Accepted in final form May 20, 1993.
This work was supported in part by a Fulbright Fellowship and a grant from the Fidia Pharmaceutical Corporation, Abano Terme, Italy, to Dr. Constantini and by National Institutes of Health Grants PO1 NS10164 and RO1 NS15590 to Dr. Young.
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