Yonsei Medical Journal
Vol. 40, No. 4, pp. 313−320, 1999

Therapeutic Time Window for Methylprednisolone in Spinal Cord Injured Rat

Do Heum Yoon[1], Young Soo Kim[1], and Wise Young[2]

Abstract

Recent clinical trials have reported that methylprednisolone sodium succinate administered within 8 hours improves neurological recovery in human spinal cord injury (SCI). Methylprednisolone, however, was ineffective and possibly even deleterious when given more than 8 hours after injury. This finding suggests that a therapeutic time window exists in spinal cord injury. In order to determine the doses, durations and timing of methylprednisolone treatment for optimal neuroprotection, a single or two bolus dose of methylprednisolone (30 mg/kg) was administered at 10, 30, 120, 150 and 240 min. after three graded spinal cord injury. The primary outcome measure was 24-hour spinal cord lesion volumes estimated from spinal cord Na^+ and K^+ shifts. A single 30 mg/kg dose of methylprednisolone at 10 min. after injury significantly reduced 24-hour lesion volumes in injured rat spinal cords. However, any other methylprednisolone treatment starting 30 min. or more after injury had no effect on 24-hour lesion volumes compared to the vehicle control group. Moreover, delayed treatment increased lesion volumes in some cases. These results suggest that the NYU SCI model has a very short therapeutic window.

Key Words: Methylprednisolone, spinal cord injury, therapeutic time window

INTRODUCTION

Many reports have indicated that progressive tissue damage occurs in traumatized spinal cord[1-5] and that treatment can significantly reduce tissue damage and improve neurological recovery when administered shortly after spinal cord injury.[6]

Several pharmacological agents have been examined and reported to be neuroprotective in a variety of animal models of spinal cord injury, but the optimum dose, duration, and timing are not known for any of the drugs, including methylprednisolone.[7,8]

Methylprednisolone is the first treatment shown to improve recovery in human spinal cord injury and it remains the only form of management shown in a Phase 3 trial to have efficacy in treating this injury.[9] Therefore methylprednisolone is regarded as the standard against which all further treatments should be compared.[10]

The beneficial effect of high-dose methylprednisolone was anticipated by many laboratory studies showing that this treatment inhibits lipid peroxidation.[11-13] Recent studies have identified lipid peroxidation as a major contributor to progressive tissue damage in injured spinal cords.[14] Methylprednisolone protects the membranes against peroxidation and thus should block the post-traumatic cascade at several sites.[12] However, it must be remembered that methylprednisolone is a glucocorticosteroid and may be acting through other mechnisms in addition to lipid peroxidation.[15]

The second National Acute Spinal Cord Injury Study (NASCIS 2) revealed that methylprednisolone, given within 8 hours after injury at a dosage of 30 mg/kg and maintained at 5.4 mg/kg/hr for 24 hours, significantly improved neurological recovery at 6 weeks, 6 months, and 1 year after injury. But treatment with methylprednisolone when initiated more than 8 hours after injury had no beneficial effect.[9] The study offers tantalizing hints that not only is the window open during the first few hours after injury, but that it slams shut within 8 hours.

Based on the pharmacodynamics of methylprednisolone, and the animal injury models, it is reasonable

Received November 4, 1998
Accepted May 26, 1999
[1]Department of Neurosurgery, Yonsei University College of Medicine, Seoul, Korea, [2]Department of Neurosurgery, New York University Medical Center, New York, U.S.A.
Address reprint request to Dr. D. H. Yoon, Department of Neurosurgery, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea. Tel: 82-2-361-5620, Fax: 82-2-393-9979, E-mail: ydoheum@yumc.yonsei.ac.kr

to postulate that the earlier after injury that drug administration begins the greater the chance of benefit.[10] Due to the relatively short-lived effect of methylprednisolone, it was suggested that, in order to maintain therapeutic concentrations in the injured spinal cord, rigorous maintenance dosing is required following the earliest possible initiation of treatment.[16]

Hall suggested that methylprednisolone therapy should be initiated as soon as possible and sustained until the intraspinal hemorrhage is resolved.[12] Two doses have also been reported to be better than a single dose.[16]

In order to determine the doses, duration, and timing of methylprednisolone treatment for optimal neuroprotection, a single or two bolus dose of methylprednisolone (30 mg/kg) was administered at 10, 30, 120, 150 and 240 min. after three graded spinal cord injury. We compared each treatment group on spinal cord lesion volume at 24 hours after injury in rats. Spinal cord contusions were induced and measured by means of a New York University weight-drop device. We quantified lesion volumes from shifts in Na^+ and K^+ levels.

MATERIALS AND METHODS

Experimental procedures

All animal protocols were reviewed and approved by NYU Medical Center Institutional Animal Care and Use Committee. A total of 289 adult male Long-Evans hooded rats weighing 400−500 gm were anesthetized with pentobarbital (60 mg/kg intraperitoneally). A catheter was placed in the femoral vein, tunneled subcutaneously to the mid-dosum where it exited the skin and another catheter was placed in the tail artery to monitor blood pressure and gases. Rectal temperatures were maintained at $37\pm0.5^{\circ}C$ with a heating pad during surgery.

The spinal cord injury was done with NYU impactor system. We checked impact velocity and the compression rate of spinal cord, which is the best predictor of 24-hour lesion volumes in contused spinal cord. Blood pressure was monitored from a catheterized tail artery. Blood gases, PH, and bicarbonate values were checked before injury.

The rats received methylprednisolone or saline vehicle starting 10 minutes after injury. Table 1 lists the treatment groups, the number of rats in each treatment-injury group and the dose and timing of each treatment protocol. All the rats were treated at 10, 30, 120, 150 and 240 minutes after injury with methylprednisolone or an equivalent volume of saline as treatment protocol.

The rats were divided into 6 groups: 1 vehicle-treated group and 5 methylprednisolone-treated groups. Methylprednisolone was given intravenously after spinal cord injury as treatment protocol. A Single or two bolus dose of methylprednisolone (30 mg/kg) was administered at various times during the first 4 hours after trauma.

At 24 hours after injury, the rats were anesthetized (60 mg/kg intraperitoneally) and then decapitated. The spinal cords were rapidly removed, frozen, and cut into 5 4-mm segments from the site of impact. One piece was centered on the impact site, 2 from the proximal cord (P1 and P2) and 2 from the neighboring distal cord (D1 and D2). Tissue lesion volumes were obtained by the previously discribed NYU method.[17]

Statistical analysis

All data were entered and initially calculated on a spreadsheet program and then transferred to a statistics program (Stat view, Super Anova 1.1 by Abacus Concepts, Berkeley, CA) for statistical analyses on a computer.

We compared individual groups treated with methylprednisolone and vehicle control group using analysis of covarience (ANCOVA) with Cr as the linear covariate. To identify groups that differed significantly from the vehicle control group, we used the

Table 1. Treatment Protocol

Group A (30)	Received 30 mg/kg of MP I.V. at 10 min. after injury
Group B (31)	Received 30 mg/kg of MP I.V. at 30 min. after injury
Group C (32)	Received 2×30 mg/kg of MP I.V. at 30 & 120 min. after injury
Group D (30)	Received 30 mg/kg of MP I.V. at 120 min. after injury
Group E (36)	Received 2×30 mg of MP I.V. at 120 & 240 min. after injury
Group F (34)	Received saline only

Fisher LSD post hoc test. ANCOVA and ANOVA were performed with commercially available statistics programs, superANOVA 1.1 and StatView 4.01 (Abacus Concepts, Berkeley, CA, USA). Regression plots were generated with StatView. All measured data are expressed as means±standard error of the means unless otherwise indicated. The criterion for significance was $p<0.05$.

RESULTS

Contusion parameters

Spinal cord contusion parameters were very consistent across treatment groups. Fig. 1 shows a scatterplot of impact velocities and spinal cord compression rates (Cd/Ct). Table 2 lists the mean impact velocities in the 12.5, 25.0 and 50.0 gm-cm injury groups. ANOVA indicated no significant differences in mean velocities and compression rates among treatment groups ($p>0.05$). Impact velocities linearly predicted spinal cord compression rates (Cd/Ct), with a correlation coefficient of 0.904.

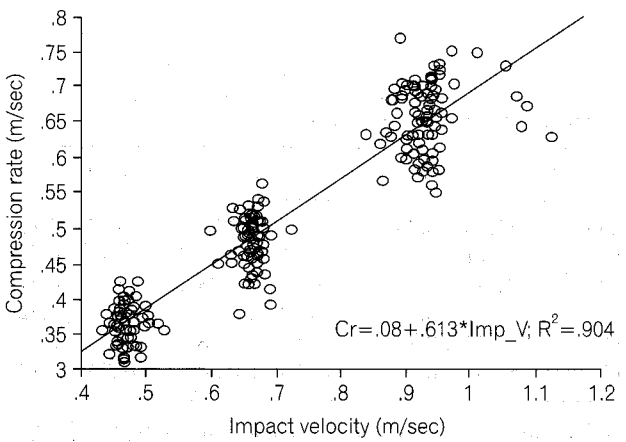

Fig. 1. Scatterplots of compression rate versus impact velocity. The regression line for all groups is shown.

Preinjury blood gases and systolic arterial pressure

ANOVA indicated no significant differences in the value of blood gases in treatment groups compared to vehicle control group except for pCO2. Table 3 lists the means of blood gas values. pCO2 in group A is different from group C ($p=0.016$), D ($p=0.058$), E ($p=0.015$), F ($p=0.005$). However, regression analysis showed no significant correlation between pCO2 and ionic lesion volumes. We could not found any statistical significance in pre-injury, injury, and post-injury systolic and diastolic blood pressure of treatment groups compared to vehicle control group.

Table 2. Contusion Parameters in Spinal Cord Injured Rats

Weight drop (gcm)	No. of rats	Impact velocity (m/sec)	Rate of cord compression
12.5	96	0.470±0.023	0.366±0.024
25.0	96	0.660±0.016	0.483±0.032
50.0	97	0.932±0.044	0.655±0.047

All values are expressed as mean±standard deviation of contusion parameters in all rats subjected to spinal cord injury and analyzed for spinal cord ionic changes.

Table 3. Preinjury Blood Gases Analysis

Group	PH	PO2	PCO2	HCO3−	B.E	O2 sat.
A	7.38±0.19E	78.96±0.59	38.04±0.82	22.93±0.47	−2.01±0.57	94.40±0.71
B	7.35±0.01	74.19±0.19	40.26±0.19	22.28±0.59	−2.59±0.57	93.11±0.75
C	7.36±0.33E	72.94±0.00	41.50±0.93	22.85±0.31	−1.11±0.48	93.02±0.54
D	7.35±0.54E	71.55±0.86	42.10±0.10	22.98±0.05	−1.70±0.56	92.00±0.91
E	7.37±0.76E	77.94±0.15	41.44±0.93	23.52±0.41	−0.96±0.50	94.32±0.43
F	7.36±0.60E	71.39±0.65	42.09±0.87	23.21±0.32	−1.32±0.39	92.00±0.92
Total	7.36±0.31E	74.52±0.99	40.97±0.41	22.98±0.37	−1.58±0.23	93.15±0.30

B.E, base excess.
All values are expressed as mean±standard deviation.

Systemic variables

All groups lost body weight after injury with mean values ranging from 5.1±2.5%. Mean blood hematocrits ranged from 36.8±5.3% (Table 4).

All the groups lost body weight after injury. ANOVA of the lost body weight and hematocrit showed significant differences between each group (lost body weight p=0.0176, hematocrit p=0.0043). Groups A, D and E significantly lost weight compared to control. Hematocrit of Groups A, C, D, E were statistically decreased compared to the contol group.

The majority of rats had gross hematuria 24 hours

Table 4. Effect of Injury and Treatment on Body Weight, Hematocrit

Group	Pre-injury wt	Post-injury wt	⊿W	Hct
A	421.30±1.04	397.97±1.78	−0.058±0.80E	0.37±0.89E
B	430.19±9.82	411.16±9.68	−0.044±0.30E	0.38±0.24E
C	450.60±1.02	429.77±0.26	−0.046±0.89E	0.35±0.010
D	448.90±0.40	424.13±1.11	−0.057±0.44E	0.37±0.011
E	457.94±2.58	432.06±2.51	−0.058±0.58E	0.35±0.51E
F	437.15±2.80	419.26±3.09	−0.043±0.76E	0.39±0.05E
Total	441.50±4.71	419.53±4.75	−0.051±0.79E	0.37±0.90E

wt, weight (g); Hct, hematocrit.
All values are expressed as mean±standard deviation.

Table 5. Effect of Injury and Treatment on Weight, Water Concentration, Tissue Na, K (1)

	P2	P1	Imp	D1	D2	Total
Wet weight	26.19±0.19	28.58±0.23	33.89±0.35	31.90±0.25	32.79±0.30	30.67±0.15
Water con.	0.68±0.27	0.70±0.21	0.76±0.49	0.71±0.31	0.70±0.90	0.71±0.48
Naw	67.15±0.25	76.23±0.48	104.50±0.43	71.87±0.37	64.13±0.23	76.77±0.41
Kw	83.93±0.27	73.94±0.52	44.86±0.45	76.22±0.39	83.76±0.24	72.54±0.42
Naw−Kw	−16.78±0.41	2.29±0.96	59.64±0.83	−4.35±0.72	−19.63±0.38	4.24±0.82
Naw+Kw	151.08±0.31	150.77±0.30	149.36±0.81	148.09±0.27	147.89±0.28	149.34±0.13

Imp, impact site; P1, P2, proximal cord; D1, D2, distal cord; wet weight (mg), tissue wet weight; Naw (umol/g), total tissue sodium concentration; Kw (umol/g), total tissue potassium concentration.
All values are expressed as mean±standard deviation.

Table 6. Effect of Injury and Treatment on Wet Weight, Water Concentration, Tissue Na, K (2)

Group	Wet wt	Water con.	Naw	Kw	Naw−Kw	Naw+Kw
A	29.73±0.31	0.71±0.81	78.14±0.33	75.01±0.34	3.06±0.64	153.22±0.34
B	30.12±0.36	0.71±0.70	76.03±0.27	72.90±0.30	3.13±0.53	148.92±0.41
C	30.23±0.40	0.71±0.73	77.49±0.29	72.18±0.25	5.31±0.50	149.67±0.40
D	31.15±0.38	0.71±0.53	78.15±0.27	71.29±0.31	6.88±0.55	149.42±0.43
E	32.03±0.38	0.71±0.26	76.83±0.19	73.10±0.20	3.73±0.36	149.93±0.35
F	30.51±0.36	0.71±0.49	76.26±0.16	72.71±0.22	3.55±0.35	148.97±0.34
Total	30.67±0.15	0.71±0.05	77.12±0.51	72.87±0.52	4.23±0.01	149.99±0.34

Wet wt (mg), tissue wet weight; Water con. tissue water concentration; Naw (umol/g), total tissue sodium concentration; Kw (umol/g), total tissue potassium concentration.
All values are expressed as mean±standard deviation.

after injury.

Mean sample wet weights were elevated at the impact site and decreased in the surrounding cord (Table 5). Additionally, wet weights increased distally toward the lumbar enlargement. methylprednisolone treatment did not decrease tissue wet weights in all treatment groups (Table 6). Moreover, group E significantly increased wet weight compared to control ($p=0.038$). To evaluate the effect of methylprednisolone on edema, tissue water concentration was calculated from 'wet weight-dry weight/wet weight'. Impact site water concentrations were greater than surrounding cord. Treatment, however, had no effect on overall spinal cord water concentration. Likewise, comparisions of individual treatment groups did not reveal any significant difference of water concentrations between groups. Wet weight (ANOVA, $p=0.0017$) and tissue water concentration (ANOVA, $p<0.0001$) were increased with injury severity.

[Na]w ($p<0.0001$) and [K]w ($p<0.0001$) changes were correlated with increasing injury severity. Injury caused a large rise in spinal cord [Na]w and a marked depletion in spinal cord [K]w, but methylprednisolone treatment had no affect on tissue [Na]w and [K]w. Although tissue [K]w was increased in group A, it did not reach significance compared to control ($p=0.1794$).

The [Na]w−[K]w increased with injury severity ($p=0.0001$), but we could not find any statistical difference between each methylprednisolone treatment group and control.

The sum of [Na]w and [K]w represents tissue ionic osmolarity. [Na]w+[K]w was reduced at the impact site and improved in adjacent segments, perhaps related to changes in tissue water concentrations. Total tissue [Na]w+[K]w was significantly elevated in group A compared to all other treatment and control groups ($p<0.0001$) (Table 6).

Drop height had very significant effects on wet weight ($p=0.0017$), tissue water concentration ($p<0.0001$), [Na]w ($p<0.0001$), [K]w ($p<0.0001$), [Na]w−[K]w ($p<0.0001$), but not on [Na]w+[K]w ($p=0.3536$).

Lesion volume assessment

ANCOVA revealed several significant treatment effects: lesion volumes in Group A were significantly smaller than those in vehicle ($p=00.0035$), Group B ($p=0.008$), Group C ($p=0.0018$), Group D ($p=0.0001$) and Group E ($p=0.0007$). But all other post-injury treatment with methylprednisolone had no significant protective effect on lesion volume compared to the control group, and two bolus injection of methylprednisolone did not provide significantly better protection against injury-induced 24-hour lesion volumes. Delayed administration of a 30 mg/kg single dose of methylprednisolone at 2 hours after injury (Group D) slightly increased lesion volumes compared to control ($p=0.0426$) (Fig. 2).

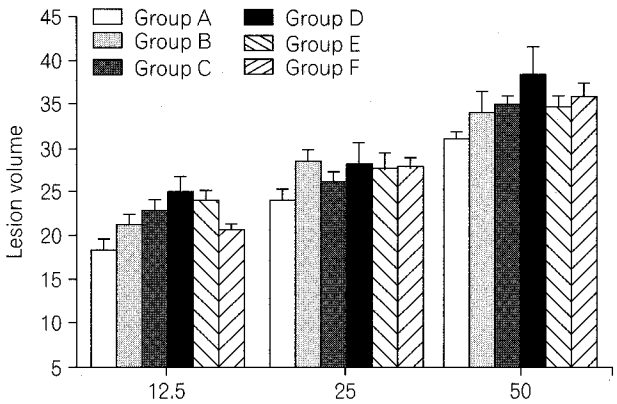

Fig. 2. Mean lesion volumes of different injury-treatment groups. Significant difference from vehicle control was only found in Group A ($p=0.0035$, ANCOVA). The error bars represent the standard error of means.

DISCUSSION

Our results showed that a 30 mg/kg dose of methylprednisolone at 10 minutes after injury significantly reduced 24-hour lesion volumes, but any other treatment with methylprednisolone starting 30 minutes or more after injury had no effect compared to the control group. These results suggest that the NYU SCI model has a very short therapeutic window.

We will first discuss some local and systemic effects of methylprednisolone and then discuss possible causes of this short therapeutic time window. Methylprednisolone treatment had no effect on overall spinal cord wet weight and water concentration. Tissue wet weights are generally believed to reflect swelling of the tissue, and water concentrations of the tissue appear to suggest tissue edema. These findings suggest that methylprednisolone had no effect on spinal cord swelling after cord injury. Lewin et al. found

that edema formation in acutely injured spinal cord is not significantly affected by glucocorticoid administration, despite an improved functional recovery and we have previously shown that tissue water concentration and edema do not correlate with injury severity, but correlate with net ionic shifts.[18]

Spinal cord contusions caused a large rise in spinal cord [Na]w and marked depletion in tissue [K]w, and methylprednisolone was well known to reduce the accumulation of sodium at the lesion site in a cat spinal cord injured model.[19,20] However, we failed to demonstrate that methylprednisolone treatment had a statistically significant effect on tissue [Na]w and [K]w. Although we found some elevation of [Na]w and [K]w in group A, these did not reach significance statistically, but total tissue [Na]w+[K]w was significantly elevated in group A. Since Na and K constitute more than 95% of tissue inorganic ions, early treatment of methylprednisolone causes elevation of tissue ionic osmolarity after injury. Our finding leads us to believe that methylprednisolone preserves the structural and functional integrity of biological membranes.

The first National Acute Spinal Cord Injury Study (NASCIS 1) began in 1979 to compare high (1 g per day for 10 days) and low dose (0.1 g per day for 10 days) methylprednisolone in human spinal cord injury.[10] Published in 1985, the study found no significant difference between high and low dose methylprednisolone started within 48 hours after spinal cord injury. The study convinced many clinicians that glucocorticoids are ineffective and raised questions about the free radical theory. By that time much evidence indicated that NASCIS 1 gave too little methylprednisolone too late.

Four major theories of secondary injury in spinal cord have emerged to explain secondary injury, emphasizing free radicals, calcium, opiates, and inflammation as causes of the progressing tissue damage.[21] These four theories are closely related and act synergistically to initiate and maintain autodestructiive mechnisms in injured spinal cords. For example, free radicals and Ca^{++} activated phospholipases operate together to break down lipid membranes.[20] Some evidence suggests that opiate receptors contribute to secondary tissue damage by increasing the release of glutamate and other neurotransmitters that open neuronal Ca^{++} channels.

Many of the data suggest that the neuroprotective effect of methylprednisolone in spinal cord injury is due to lipid peroxidation inhibition.[22]

The optimal dose of methylprednisolone required for lipid peroxidation inhibition was 30 mg/kg, substantially greater than doses used in NASCIS 1. Methylprednisolone preserved microvasculature, reduced metabolic derangements, prevented post-traumatic ischemia and restored extracellular calcium ion activity and improved neurological recovery in animal spinal cord models.[7,23] The methylprednisolone dose-response curve, however, is bell-shaped. Doses of 15 mg/kg were less effective and doses of 60 mg/kg were not only ineffective but paradoxically deleterious. A single intravenous dose markedly decreased lactate accumulation and loss of pyruvate, but 15 and 60 mg/kg doses were ineffective. Likewise, 30 mg/kg significantly improved blood flow in injured cat spinal cords, but 60 mg/kg was ineffective.[21]

Hall et al. colleagues have hypothesized that methylprednisolone protects neurons by scavenging oxygen free radicals.[12] This hypothesis is based on the observation that neuroprotective doses of methylprednisolone greatly exceed those required for glucocorticoid receptor activation and are close to the doses that inhibit lipid peroxidation in injured spinal cords.

Methylprednisolone, however, is a glucocorticoid with potent anti-imflammatory properties. Glucorcorticoids induced synthesis and a release of anti-inflammatory peptides, including lipocortins that inhibit calcium activated phospholipase activity by binding to membrane phospholipid substrates. As well, methylprednisolone is a potent immunosuppressive and anti-inflammatory drug that inhibits phospholipase A2 activity, alters neuronal excitability, and improves post-traumatic spinal cord blood flow.[15]

In 1990, the second National Spinal Cord Injury Study (NASCIS 2) showed that very high doses of methylprednisolone significantly improve motor and sensory recovery if given within 8 hours after spinal cord injury. However, methylprednisolone was not only ineffective when started more than 8 hours after injury, but it have made have been deleterious.[9] This finding suggests that a therapeutic time window exists in spinal cord injury.

Spinal tissue uptake of methylprednisolone decreased rapidly with time after injury. This is perhaps due to secondary post-traumatic tissue loss and to a progressive decrease in blood flow to the injury site.[23] However, a more important factor in predicting a

limited therapeutic time window is the fact that secondary tissue degeneration evolves rapidly after injury and that this process, for the most part, is irreversible.

The optimal dose of glucocorticoid is not known, nor has the best therapeutic regimen been developed. Hall has suggested that frequently repeated maintenance doses are necessary in order to maintain blood flow, tissue preservation, and to maximize the potential for recovery.[12]

Many designers of therapeutic protocols seldom consider the possibility that "secondary injury mechnisms" may serve a protective, clean-up, or recovery purpose.[24] For example, lipid peroxidation and Ca activated phospholipase activity are likely to be important for rapid breakdown of moribund cells to release Ca^{++} binding substances that lower extracellular Ca^{++} and protect surviving cells. Methylprednisolone rapidly increases white matter blood flow in injured spinal cord [25] and also prevents the delayed fall of extracellular Ca^{++} at the injury site. These findings suggest that very high doses of methylprednisolone facilitate lipid peroxidation and thereby would be deleterious. Finally, the therapeutic window may vary with injury severity and both the time course and intensity of lipid peroxidation are likely to change with injury severity.[21]

In NASCIS 2, the 8-hour period was simply the median treatment time which conveniently segregated the patient population into equal groups of early and late treatments for analysis.[9] Therfore, the optimal therapeutic time for methylprednisolone in human spinal cord injury is likely to be shorter than 8 hours.

In cats, methylprednisolone has been shown to be effective when given as late as 45 minutes after injury.[21]

Our results, however, suggest that the therapeutic time window for methylprednisolone is less than 30 minutes after contusion. Two possible explanations should be considered for the short therapeutic time window in rats. First, recent studies suggest that rats very rapidly restore extracellular ionic levels after injury.[26]

Whereas extracellular potassium cleared from the cat spinal cord with a half life of 45 min, in the rat, the mean half time was only 11 min. Clearance may have been faster in the rat due to the smaller volume of tissue in which extracellular potassium was elevated compared to a human or cat, a point emphasized by Cordingley and Somjen.[27] [K]e returned to near physiological levels after approximately one hour. Earlier studies in cats showed that K^{+} restored to baseline levels only after 1−2 hours while Ca^{++} did not return to pre-injury levels for more than 4 hours. Rats, by contrast, recover their K^{+} & Ca^{++} levels within 10 minutes and 30 minutes respectively.[24]

We had earlier proposed that the profound and prolonged fall in extracellular Ca^{++} at the injury site may be neuroprotective.[24] If so, methylprednisolone should be given before extracellular Ca^{++} is restored to pre-injury level and this may explain the short therapeutic time window for methylprednisolone in rats. A second explanation for the short therapeutic time window may simply be the rapid metabolism of rats. In general, rats have much shorter plasma drug half-lives, faster development of necrotic lesions, and higher blood flows. Secondary injury processes such as cytokine release, neurotransmitter release, blood flow changes, lipid peroxidation, and others may occur much faster in rats. Consequently, methylprednisolone must be given earlier after injury.

In conclusion, the finding of a short therapeutic time window in the rat spinal contusion model has important implications. The finding would explain negative treatment studies where a drug is administered more than 30 minutes after injury. Further treatment studies, especially those involving methylprednisolone, should include at least one treatment arm delivered shortly after injury.

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