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Methylprednisolone treatment of experimental spinal cord injury

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Lipid peroxidation has been identified as a deleterious consequence of contusive spinal cord trauma and of thermal injury. The L3–L6 spinal cord segment was thermally injured using a radiofrequency heating chamber mounted on the vertebral column of anesthetized rats. Hind limb function was assessed 2 hours later. A bolus of methylprednisolone (MP, 30 mg/kg) was then given intravenously, followed by 5.4 mg/kg/hr MP for 6 or 24 hours. Cord water content and regional spinal cord blood flow (RSCBF, ^{14}C-butanol distribution) were measured at seven cord levels after function had been reassessed following treatment. Untreated rats were given vehicle. The study was randomized and blinded. *Results*: Edema in heated segments was progressive over 24 hours, but was the same in treated vs untreated rats. RSCBF in heated segments was the same in treated vs uninjured controls at 6 and 24 hours. In untreated rats, RSCBF in the heated segment was elevated by 30% at 6 hours, but was the same as uninjured control by 24 hours. In the unheated segments of untreated rats, RSCBF was elevated at 24 hours. At 24 hours, RSCBF was lower in treated vs untreated rats at all levels, including the heated one. Limb function deteriorated equivalently in both groups. *Conclusion*: MP obviated the early rise in RSCBF in heated segments and the elevations in RSCBF in uninjured segments, but had no effect on cord edema or on limb function.

Keywords: radiofrequency heat; regional blood flow; spinal cord edema; ^{14}C-butanol distribution; methylprednisolone.

Introduction

Glucocorticoids have been used clinically to treat acute spinal cord syndromes of diverse etiology, including trauma. Experimentally, there is evidence that glucocorticoids in appropriate dose are beneficial, at least in some of those circumstances.[1,2] A recent clinical trial showed that patients with spinal cord injury had improved function provided they were treated with methylprednisolone (MP) in appropriate dose for 23 hours beginning within 8 hours of injury.[3] The mechanisms of the protective action of glucocorticoids in injured or abnormal neural tissues are presently incompletely understood. Prominent among those mechanisms suggested by the experimental evidence is an effect on regional blood flow that may be mediated either directly, by altering vascular tone; or indirectly, for example, by reducing the local generation of vasoactive substances.[4] Another mechanism, the reduction of edema, is less widely favored than it was previously, based on recent experimental evidence.[5,6]

At the molecular level, considerable evidence suggests that local tissue lipid peroxidation is an important injurious consequence of spinal cord contusion.[7,8] Methylprednisolone may be beneficial in cord injury because of its antioxidant properties.[3,9] Lipid peroxidation also occurs locally immediately after burn injury; and antioxidant therapy has benefit in experimental burns.[10,11] A model of cord injury which does not mechanically disrupt blood vessels or axons would therefore be potentially advantageous for the study of those

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secondary phenomena which are shared by both contusive and thermal tissue injury mechanisms.

In these experiments, we examined the short term effects of high dose MP on regional spinal cord blood flow (RSCBF) and on segmental cord edema formation in rats in which a short segment of the lumbar spinal cord had been heated briefly by the passage of radiofrequency (RF) current. The short-term functional neurological status of the hind limb was assessed during the first 24 hours after injury using two clinical scoring systems previously reported.[12,13]

Materials and methods

Induction of spinal cord injury

Male Sprague-Dawley rats weighing from 323 to 480 g were used. Fluothane 1.5% inhalation anesthesia was administered after intraperitoneal pretreatment with lactated Ringer's solution, 20 ml/kg, and 0.004 mg/kg atropine subcutaneously. The fur was clipped from the lower belly wall, around the neck and from the upper posterior torso. A cystostomy was made through a small suprapubic incision using a Silastic catheter (0.085 in OD, Dow Corning) to prevent bladder overdistention and hemorrhage. A plastic Vialon catheter (1.1 mm OD, Deseret Medical) was inserted into the left jugular vein through a short cervical incision.

Through a short dorsal incision, the T12–L2 spinous, accessory, mammillary (or lateral) and transverse processes were exposed by dissecting the surrounding muscles. A 1 mm laminectomy was performed at T12 using a power drill with a burr bit. The intact dura was carefully depressed from the bone by blunt dissection and a 0.225 mm diameter thermocouple microprobe (IT-23, Sensortek, Inc, time constant 0.005 second) was inserted through the laminectomy into the epidural space and advanced caudally so that its tip was at the T13–L1 vertebral interspace. The microprobe was connected to a digital thermometer (BAT-8, Bailey Instrument Co).

A U-shaped clear plastic chamber, the parallel limbs of which were 15 mm apart, tabricated to contain 8 × 5 mm copper electrodes wired to a RF generator (Henry Company, Model 100D) was inverted and positioned over the exposed spinal column at the T12–L1 level. The chamber, positioned within the cavity created by the muscle dissection, was filled with lactated Ringer's solution warmed to 37 °C.

Radiofrequency current (2 MHz) was then passed through the 5 mm in length segment of spinal column and spinal cord contained within the electrodes by manipulating the power output of the RF generator. The desired temperature within the spinal canal (47 °C) was reached within 40–80 seconds. Temperatures were recorded at 20-second intervals. The temperature within the heated segment was maintained for 10 minutes, after which the wound was irrigated with room temperature lactated Ringer's solution. The chamber was then removed, the position of the thermocouple microprobe confirmed and the wound sutured. The fluothane anesthesia was discontinued.

Randomization of treatments

Provided that the hind limb neurological function 2 hours following injury was appreciably impaired, as evident from a neurological impairment score (NIS, see below) of between 6 and 12, the rats were randomly assigned by lottery to one of the control or treatment groups. Approximately 50% of rats were sacrificed at this stage by pentobarbital overdose because they did not meet this criterion. Test rats, individually caged, were treated with intravenous MP sodium succinate in lactated Ringer's solution beginning 2 hours after injury with a bolus injection of 30 mg/kg of a solution containing 2 mg/ml. In rats randomized for study at 24 hours, a continuous infusion of MP (3 mg/ml) at the rate of 5.4 mg/kg/hr was instituted 1 hour later using a syringe pump (Model 355, Sage Instruments). In rats randomized for study at 6 hours, a second bolus of MP, 16.2 mg/kg was given 1 hour later (5.4 mg/kg × 3 hr). Control rats were given equivalent volumes of lactated Ringer's solution. All infusions were continued until further studies were performed. Rats

studied after 24 hours were given the MP through the jugular venous catheter, which was tunnelled to exit the skin at the nape of the neck and attached to a swivel/spring device to prevent dislodgement.

Additional controls

In 12 other rats, measurements of spinal cord water content ($n = 6$) and of RSCBF ($n = 6$) were performed 2 hours following laminectomy alone and after a normal neurological status had been verified. These rats served as uninjured controls (group 1). The same two measurements were also done in 12 rats that had undergone cord injury 2 hours previously and whose neurological scores using the NIS were also within the range 6–12 (group 2). This group was included to determine a pretreatment baseline for comparison to data obtained later following injury.

Measurements at 6 or 24 hours

The rats were anesthetized with a single intraperitoneal injection of pentobarbital, 50 mg/kg, following pretreatment with 0.004 mg/kg atropine subcutaneously and 10 ml/kg of lactated Ringer's solution intravenously.

Through small cervical and femoral incisions, plastic Vialon catheters were inserted into the right carotid artery and the right jugular vein (1.1 mm OD) and the left femoral artery (0.7 mm OD). The carotid catheter tip was in the ascending aorta, that of the jugular catheter at the superior vena cava, that of the femoral catheter at the external iliac artery. Mean arterial blood pressure (MAP) was measured by connecting the femoral catheter to a Statham 23 ID transducer positioned at heart level and a Tektronix Model #414 pressure monitor. Arterial blood PaO_2, $PaCO_2$ and pH were determined immediately prior to the performance of blood flow measurements using an Instrumentation Laboratory System 1301 blood gas analyzer. Rectal temperature was monitored and maintained near 37 °C using either a heating blanket or an infrared lamp. The interval between pentobarbital administration and the subsequent measurement of either RSCBF or of spinal cord water content was controlled.

Regional blood flow

Regional blood flow in various levels of the spinal cord was measured using the ^{14}C-butanol 'indicator-fractionation' method we have described previously.[14] Twenty-five μCi of ^{14}C-butanol were rapidly injected (less than 1 second) through the jugular catheter 5 seconds after a constant rate of arterial hemorrhage had been visually verified from the previously declamped femoral catheter. Fifteen seconds after the indicator had been injected, 1.0 ml of cyanoacrylate glue was injected into the carotid catheter. This resulted in the formation of a dense intraluminal coagulum which extended from the aortic valve to beyond the aorto-iliac bifurcation and arrested blood flow near-instantaneously. Simultaneously, the femoral catheter was clamped and the rat was killed with concentrated intravenous KCl.

An extensive laminectomy was immediately performed. Seven segments of the spinal cord were procured at vertebral levels: C3–C5, T3–T5, T7–T9, T12, T13–L1 (heated level), L2–L3 and L4–L6, which represent the vertebral levels of spinal cord corresponding to spinal cord levels C3–C5, T3–T5, T7–9, L1–L2, L3–L6, S1–S4 and cauda equina, respectively. These samples were solubilized for 48 to 72 hours and their radioactivity determined by scintillation spectrophotometry. Regional spinal cord blood flow was calculated using the equation: $F_t = (Q_t F_a)/(Q_a M_t) \times 100$ where:

- F_t is regional blood flow (ml min^{-1} 100 g^{-1})
- F_a is rate of external hemorrhage (ml min^{-1})
- Q_t is indicator content in the tissue (cpm g^{-1})
- Q_a is indicator content in the arterial blood (cpm g^{-1})
- M_t is sample weight (g).

The theoretical basis and validation of this methodology and the derivation of this equation from the Fick principle is given in detail in previous publications.[14,15]

Measurements of spinal cord water content
These rats were killed by pentobarbital overdose. An extensive laminectomy was performed immediately. Seven segments of the spinal cord were procured at the same levels as those obtained for the RSCBF measurement. These samples were weighed immediately (wet weight), then oven-dried to constant weight for at least 24 hours. Water content (WC) was calculated from the formula % WC = (wet weight − dry weight)/wet weight 100.

Clinical neurological function
Assessment of the functional status of the hind limbs and tail was carried out by an experienced observer blinded to the treatment proposed or in progress at 2 hours after injury, prior to initiating treatment in all rats and also immediately before the performance of physiological measurements at either 6 or 24 hours after injury.

This assessment consisted of (1) a neurological assessment score (NIS), modified slightly from LeMay *et al* and outlined in Table I (normal = < 3);[16] (2) a corrected, combined behavioral score (CCBS) as reported by Gale *et al*[12] and later modified by Kerasidis *et al*[13] (Table II). Scores from each hind limb were recorded separately. The two scoring systems were used with the aim of minimizing the appreciable inherent error in evaluating neurological function in experimental animals.

Physiological variables
Body weight, rectal temperature and pentobarbital anesthetic times were recorded. Measurements of RSCBF were immediately preceded by determinations of the MAP and of arterial blood PaO_2, $PaCO_2$ and pH.

Data analysis
All data are given as the mean and standard error of the mean. Parametric data were analyzed on the Washington University main frame computer by analysis of variance followed by the use of statistical contrasts. The nonparametric data resulting from the neurological scoring systems described were analyzed using the Wilcoxon rank sum test. An unpaired *t* test was used when appropriate. The null hypothesis was rejected at $p < 0.05$.

Table I Neurological impairment score (NIS)

Test	Points
Walking with hind limbs (HL)	
No evidence of deficit	0
Toes flat under body when walking but ataxia exists	1
Knuckle walks	2
Movement of HL but unable to knuckle walk	3
No movement, drags HL	4
Horizontal rope	
Grasps rope and pulls up with HL	0
Raises HL and grasps rope without pulling	1
Raises HL but cannot grasp rope	2
Does not raise HL	3
Screen	
HL grasp screen[a] to 180° for more than 5 s	0
HL grasp screen to 180° for less than 5 s	1
HL grasp screen past vertical but not to 180°	2
HL fall from screen past vertical (270°–180° range)	3
HL fall from screen before vertical (0°–270° range)	4
Pain sensation (HL)	
Withdrawal to toe pinch	0
Squeals to toe pinch but does not withdraw	1
No reaction to toe pinch	2
Pain sensation (Tail)	
Withdrawal to tail pinch	0
Squeals to tail pinch but does not withdraw	1
No reaction to tail pinch	2

[a]A plastic screen with 1.2 × 1.2 cm apertures. The round screening is 2 mm in diameter. Normal < 3, maximum 15.

Results

Spinal cord water content (Table III, Fig 1)
Two hours after injury, cord water content was unchanged at all levels compared to the uninjured controls.

By 6 hours after injury, the water content

Table II The combined behavioral score and its correction (CCBS)

Test	Points	Motor score	Correction
Motor score		5	+10 points for any two:
Normal walking	0		Abnormal swim test
Walks with only mild deficit	5		Abnormal pain withdrawal
Hind limbs (HL) can support weight	15		Inclined plane score $\leq 40°$
Frequent/vigorous movement in HL, no weight bearing	25	15	−10 points:
Barely perceptible movement in HL, no weight bearing	40		Hot plate response lacenty < 60
No movement in HL, no weight bearing	45		+10 points for any two:
Toe spread			Inclined plane score $\leq 30°$
Normal full toe spread	0		Abnormal toe spread response
Partial spreading of toes	5		Abnormal extension withdrawal
No spreading of toes	5	25	−10 points:
Righting			Inclined plane score $\geq 40°$ or
Normal righting counter to direction of the roll	0		Swim test is normal
Weak/delayed attempt or rights in the direction of the roll	5		+15 points:
No attempt to right itself	5		Inclined plane score $< 30°$ and
Withdrawal reflex (extension, pressure, pain)			Righting is abnormal
Normal withdrawal	0	40	−15 points:
Weak withdrawal	15		Pressure/pain withdrawal is normal
No withdrawal	15	45	−5 points:
Hyperactive withdrawal	15		Paw placement or extension withdrawal normal
Hot plate			Pressure/pain withdrawal is normal
Hind paw licking in less than 30 seconds	0		
> 60 seconds	5		
Paw placement when held at right angles to table top			
Normal placement	0		
Weak attempt to place paw	5		
No attempt to place paw	5		
Swim–climb testing			
Normal swimming and climbing using hind limbs	0		
Abnormal swimming and climbing without using hind limbs	5		
Inclined plane			
Maintains position for 5 s at angle of 50°–60°	0		
Maintains position for 5 s at angle of 45°	5		
Maintains position for 5 s at angle of 35°–40°	10		
Maintains position for 5 s at angle of $\leq 30°$	15		

Table III Spinal cord water content (% wet weight)

Group	Injury level C3–C5	T3–T5	T7–T9	L1–L2	L3–L6	S1–S4	Cauda equina
(1) Uninjured control (Laminectomy only, $n = 6$)	68.8 ± 0.2	68.1 ± 0.1	68.4 ± 0.3	69.0 ± 0.3	70.1 ± 0.2	69.8 ± 0.2	67.9 ± 0.2
(2) 2 hours after injury Untreated ($n = 6$)	69.0 ± 0.3	68.2 ± 0.3	68.0 ± 0.3	68.9 ± 0.7	71.2 ± 0.7	69.4 ± 0.2	66.7 ± 0.5
(3) 6 hours after injury Untreated ($n = 8$)	68.8 ± 0.4	68.3 ± 0.4	68.6 ± 0.5	70.5 ± 0.3	73.6[1] ± 0.4	70.2 ± 0.6	67.4 ± 0.4
(4) 6 hours after injury MP[a]-treated ($n = 8$)	69.4 ± 0.2	68.7 ± 0.3	69.7 ± 0.3	71.4[2] ± 0.3	73.8[1] ± 0.6	70.3 ± 0.3	68.3 ± 0.4
(5) 24 hours after injury Untreated ($n = 6$)	69.3 ± 0.3	69.5[3] ± 0.6	69.8 ± 0.7	72.0[4] ± 1.0	74.5[1] ± 0.4	70.5 ± 0.8	68.4 ± 0.5
(6) 24 hours after injury MP-treated ($n = 6$)	69.5 ± 0.3	69.5[3] ± 0.6	69.7 ± 0.8	72.7[5] ± 1.1	75.5[1] ± 1.0	71.0 ± 0.6	68.8 ± 0.6

[a]Methylprednisolone.
p vs uninjured control: [1] = 0.0001; [2] = 0.01; [3] = 0.03; [4] = 0.004; [5] = 0.0004.

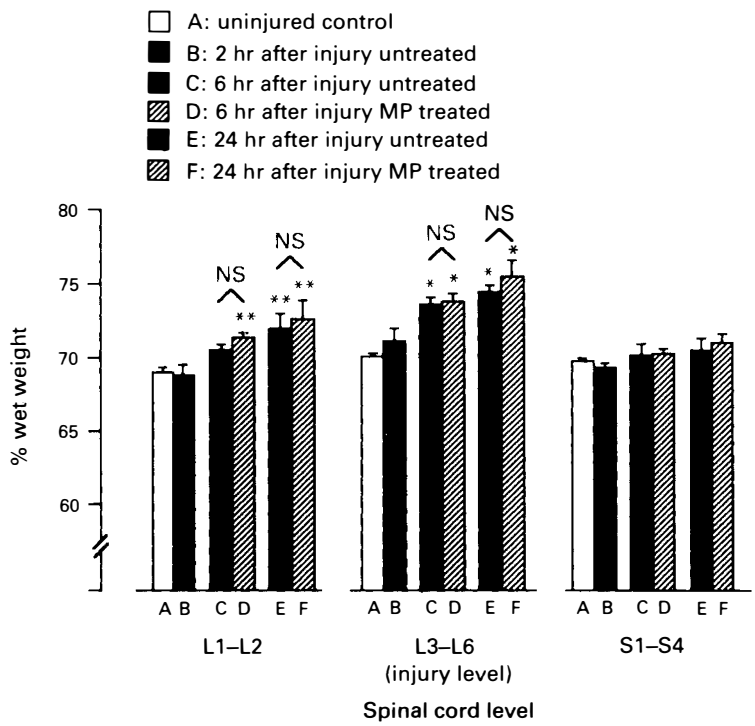

Figure 1 The changes in water content in the injured and adjacent spinal cord segments. Vs A: $^{*}p < 0.0001$; $^{**}p < 0.01$.

of the heated L3–L6 segment was increased compared to uninjured control in both the treated and untreated rats, but water content did not differ in treated vs untreated rats. In the L1–L2 cord segment adjacent cephalad to the heated one, water content was increased compared to the uninjured controls in the treated rats but not in the untreated ones.

Twenty-four hours after injury there was still no difference in water content in treated vs untreated rats in the heated L3–L6 segment, although in both groups the water content of this segment was more than that of the uninjured controls. In both groups, edema was now also evident in the adjacent L1–L2 segments and was similar in amount. There was a smaller, also equivalent increase in water content in the T3–T5 segment of both the treated and untreated rats.

Regional blood flow (Table IV, Figs 1,2)
By 2 hours after injury, RSCBF was elevated in untreated group 2 rats compared to uninjured control by from 24 to 35% at all cord levels, excepting only the cauda equina. Although pentobarbital anesthesia depresses RSCBF,[4] this elevation was present despite the brief duration of anesthesia in this group.

By 6 hours after injury, RSCBF had returned to normal at all levels in untreated rats except at the heated L3–L6 segment, where it remained elevated at a value nearly identical to that present at 2 hours. In treated rats, however, RSCBF was no different from that in the uninjured controls either in the heated L3–L6 segment or in all the other segments cephalad to it. In the S1–S4 segment of the treated rats, RSCBF was depressed compared to that in the uninjured controls.

By 24 hours after injury, RSCBF in treated rats was lower than in the untreated rats at all levels of the cord. In the heated L3–L6 segment, RSCBF at 24 hours in treated rats was normal compared to uninjured control, but was less than that in untreated rats. Regional spinal cord blood flow was less than that of the uninjured controls in both the L1–L2 and S1–S4 segments adjacent to the heated one and in the C3–C5 segment. In the untreated rats, RSCBF was elevated compared to uninjured control at all unheated cord levels, the difference reaching significance only at C3–C5, T3–T5, T7–T9 and in the cauda equina.

Neurological function (Table V)
The uninjured controls had normal neurological function. At 2 hours following injury, both the NIS and the CCBS were clearly abnormal in the group 2 rats. There were no intragroup differences using either scoring system or when the scores from each of the hind limbs were compared separately. At 6 hours, there were no intergroup differences, nor was there a difference from the 2-hour scores in either the treated or untreated rats. At 24 hours, however, deterioration of function was present in both treated and untreated rats, but, as at 6 hours, there were no differences between groups.

Physiological variables (Table VI)
In the untreated rats at 24 hours (group 5), MAP was lower than in treated rats (group 6). The duration of pentobarbital anesthesia was less in the untreated group 2 rats than in the other groups. Otherwise, there were no intergroup differences that were judged to be of physiological significance.

Discussion

There is no ideal experimental model of spinal cord trauma. The clinical spectrum of cord injury is in itself diverse.[4] Much spinal cord trauma research is based on the concept, backed by considerable experimental evidence, that the ultimate functional status is importantly influenced by secondary phenomena that may occur simultaneously or sequentially during minutes, hours or days after injury. The improvement in neurological function documented in the clinical trial of MP treatment already alluded to supports that concept and provides as well another important reason to investigate the mechanisms of MP action in the posttraumaic period.

One of the most widely held concepts and one supported by considerable evidence

Table IV Regional spinal cord blood flow (ml min^{-1} 100 g^{-1})

	Spinal cord level						
				Injury level			
	C3–C5	T3–T5	T7–T9	L1–L2	L3–L6	S1–S4	Cauda equina
Group							
(1) Uninjured control (Laminectomy only, (n = 6)	62.3 ± 3.1	50.4 ± 1.3	48.0 ± 1.5	59.6 ± 3.3	62.9 ± 4.0	50.4 ± 2.6	28.6 ± 1.8
p, 1 vs 2	0.0003	0.005	0.0023	0.005	0.0085	0.004	
(2) 2 hours after injury Untreated (n = 6)	84.3 ± 5.0	65.1 ± 4.6	63.0 ± 3.6	79.1 ± 3.3	81.8 ± 5.5	38.2 ± 3.2	31.4 ± 0.87
(3) 6 hours after injury Untreated (n = 8)	61.6 ± 2.8	52.9 ± 2.2	53.8 ± 2.1	65.7 ± 2.4	82.5[1] ± 3.3	46.2 ± 1.6	37.1 ± 3.2
p, 3 vs 4					0.03		
(4) 6 hours after injury MP[a]-treated (n = 8)	60.7 ± 3.1	51.2 ± 2.3	52.5 ± 3.4	59.4 ± 3.9	69.4 ± 4.4	41.9[2] ± 2.5	36.6 ± 2.3
(5) 24 hours after injury Untreated (n = 6)	80.0[3] ± 6.5	69.8[4] ± 6.1	69.7[5] ± 4.7	69.3 ± 3.9	64.4 ± 6.2	57.0 ± 2.6	51.8[5] ± 5.5
p, 5 vs 6	0.0001	0.0001	0.0001	0.0001	0.048	0.0001	0.0003
(6) 24 hours after injury MP-treated (n = 6)	46.6[6] ± 1.5	44.3 ± 1.4	45.4 ± 1.7	46.2[7] ± 3.4	50.5 ± 4.0	39.8[7] ± 3.7	33.1 ± 3.1

[a]Methylprednisolone.
p vs uninjured control: [1] = 0.0039; [2] = 0.03; [3] = 0.003; [4] = 0.0003; [5] = 0.0001; [6] = 0.008; [7] = 0.013.

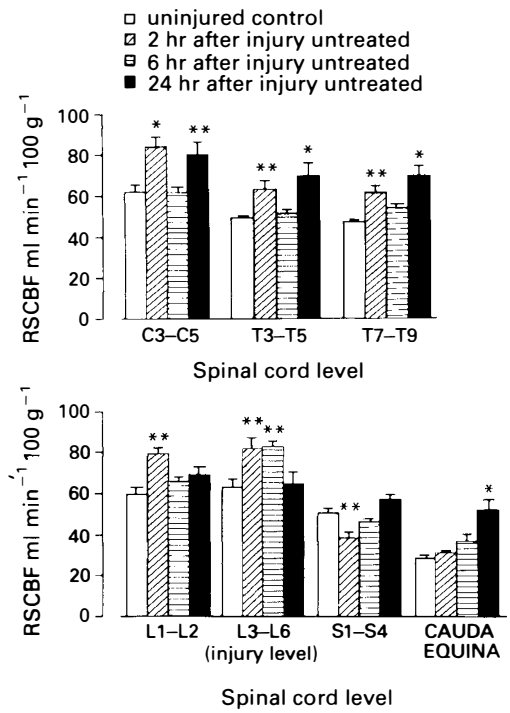

Figure 2 The changes in RSCBF in uninjured control and in injured, untreated rats. Note the tendency to biphasic elevation of RSCBF at 2 and 24 hours after injury in the three most cephalad cord segments. Vs uninjured control: $^{*}p < 0.001$; $^{**}p < 0.01$.

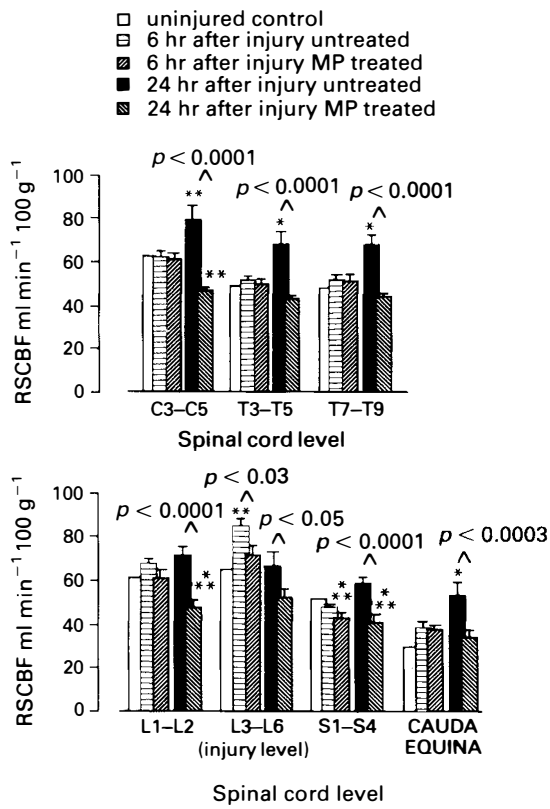

Figure 3 The effect of methylprednisolone treatment (MP) on RSCBF. Vs uninjured control: $^{*}p < 0.001$; $^{**}p < 0.01$; $^{***}p < 0.05$.

is that spinal cord ischemia contributes importantly to the damage caused by trauma, noting in this regard the important distinction between ischemia, which connotes tissue metabolic impairment, and mere hypoperfusion.[17–20] A partial list of other secondary phenomena in addition to ischemia includes the generation of eicosanoids,[21,22] free radicals,[1] the induction of lipid peroxidation[23] and intracellular ionic perturbations.[2] The time course, relative importance and the interactions among these factors, which are potentially amenable to therapy, are presently largely unknown or at best incompletely understood.

The mechanism of injury in the present model is clearly dissimilar to that usually occurring clinically, excepting perhaps in high voltage electrical injury. This model also differs in several significant aspects from those most often used previously, namely impact or compression, but it is not associated with spinal shock or respiratory dysfunction and avoids mechanical disruption or occlusion of large blood vessels and neural structures. Also, major changes in MAP do not occur during or immediately following injury with the time-temperature coefficient used here.

There is consensus that the tissue injury resulting from the passage of radiofrequency current is the result of the heat generated.[24,25] In the present model, progressive deterioration of neurological function occurred (Table V). The model therefore permits the investigation of secondary phenomena which appear, at least in part, to be shared, as lipid peroxidation is operative after both contusive spinal cord trauma and thermal injury to non-neural soft tissue.[7,26] We do not yet have biochemical evidence supporting a role for

Table V Neurological function

Group	Time after injury					
	2 hours		5 hours		23 hours	
	NIS[a]	CCBS[b]	NIS	CCBS	NIS	CCBS
(1) Uninjured control (Laminectomy only, (n = 12)	1.8	0				
(2) 2 hours after injury Untreated (n = 12)	8 ± 1.0	58 ± 8.0				
(3) 6 hours after injury Untreated (n = 16)	8 ± 0.4	57 ± 3.0	9 ± 0.5	67 ± 4.0		
(4) 6 hours after injury MP[c]-treated (n =16)	8 ± 0.5	58 ± 5.0	9 ± 0.8	63 ± 6.0		
(5) 24 hours after injury Untreated (n = 12)	8 ± 0.6	58 ± 5.0			13[1] ± 0.4	94[1] ± 2.0
(6) 24 hours after injury MP-treated (n = 12)	7 ± 0.6	53 ± 6.0			12[1] ± 1.0	84[1] ± 7.0

[a]Neurological impairment score.
[b]Corrected combined behavioral score.
[c]Methylprednisolone.
[1] = $p < 0.01$ vs 2 hours after injury.

oxidative injury in the model described here, but the probability seems virtually certain, considering the numerous reports that have appeared supporting a role for oxidative injury after thermal burns, only some of which are cited in this report.[27–29]

After contusive cord injury, tissue levels of the lipid peroxidation end-product malondialdehyde increase and cholesterol oxidation products appear; there are decreased levels of antioxidants such as α-tocopherol and reduced ascorbate.[7] Following burns, local and circulatory levels of malondialdehyde also increase and antioxidant therapy with allopurinol or ascorbic acid reduces local edema formation.[10,11]

In the present experiments, MP had no perceptible effect on the rate of early edema formation, either in the heated cord segment where edema was greatest or in any of the other cord segments examined. This finding agrees with one carefully controlled study in an impact injury model in which administration of glucocorticoid before or after injury did not reduce edema formation.[5] It is important to note, however, that despite the absence of an effect of corticosteroid treatment in either reducing cord edema or the rate at which it resolved, there was significantly more functional recovery observed in steriod-treated subjects during the 6 day follow up period.[5,6]

Treatment with MP had a definite effect on RSCBF, however, which was evident both at the injury site and elsewhere in the cord. Specifically, MP eliminated the 30% increase in RSCBF in the heated segment that was present after 6 hours in untreated rats and was also present 2 hours after injury, prior to the initiation of treatment. Further, by 24 hours, although RSCBF in the heated L3–L6 segment was at the preinjury level in both the treated and untreated groups, RSCBF in the treated group was lower than that in untreated rats in that segment. In other words, RSCBF in the heated segment of treated rats was invariably less than that in the untreated rats and was never different from that in uninjured controls. Also, the tendency toward elevation of RSCBF in uninjured cord segments after 24 hours was not observed in the treated rats, in which RSCBF was less than that in the untreated rats at all levels and

Table VI Physiological variables[a]

Group	MAP[b] (mmHg)	Rectal temp (°C)	Body weight (g)	Pentobarbital anesthesia duration (min)	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	pH
(1) Uninjured control (Laminectomy only, $n = 6$)	123 ± 6	36.6 ± 0.1	428 ± 15	60 ± 9	80.5 ± 2.0	38.8 ± 2.6	7.38 ± 0.02
(2) 2 hours after injury Untreated ($n = 6$)	119 ± 6	37.2 ± 0.2	428 ± 13	34[1] ± 2	89.2 ± 11.1	36.4 ± 2.3	7.37 ± 0.02
(3) 6 hours after injury Untreated ($n = 8$)	84 ± 7	37.7 ± 0.2	386 ± 17	61 ± 1	94.2 ± 10.5	32.6 ± 0.8	7.40 ± 0.02
(4) 6 hours after injury MP[c]-treated ($n = 8$)	81 ± 3	37.3 ± 0.1	380 ± 11	57 ± 1	95.9 ± 8.4	34.0 ± 0.6	7.37 ± 0.02
(5) 24 hours after injury Untreated ($n = 6$)	65[2] ± 2	37.1 ± 0.2	392 ± 6	60 ± 2	70.2 ± 3.7	36.8 ± 1.0	7.37 ± 0.01
(6) 24 hours after injury MP-treated ($n = 6$)	82 ± 4	37.3 ± 0.1	366[3] ± 8	58 ± 2	82.5 ± 5.5	33.5 ± 1.3	7.42[3] ± 0.01

[a]Data from rats in which RSCBF was measured.
[b]Mean arterial blood pressure.
[c]Methylprednisolone.
[1] $p < 0.01$ vs all other groups; [2] $p < 0.01$ vs 6; [3] $p < 0.05$ vs 5.

was lower than in the uninjured controls in the C3–C5, L1–L2 and S1–S4 segments.

These data tend to confirm prior experimental evidence, obtained mainly from impact or compression models and using other methods of measuring RSCBF, indicating that high dose MP as used here affects RSCBF following injury.[2,30] When injury was severe in those models (as it was here, as evidenced by the 24-hour neurological scores) MP was found to blunt or prevent an early fall in RSCBF.[2] In the present model, however, RSCBF was elevated at 2 and 6 hours after injury in the injured segment of the untreated rats and there was an apparent biphasic elevation in flow at 2 and 24 hours in most of the other, unheated segments.

In untreated rats, the directional changes in RSCBF were not necessarily similar in heated and uninjured segments. At 2 hours, RSCBF was elevated vs uninjured control by from 24 to 35% at all cord levels cephalad to and including the heated site, but was depressed at S1–S4 and unchanged in cauda equina. At 6 hours, RSCBF in the heated segment of untreated rats was still elevated by about 30%, but flow in all remaining cord segments was then the same as in the uninjured controls. At 24 hours, RSCBF in all uninjured cord segments was elevated, while flow in the heated segment did not differ from uninjured control. The elevated flow in uninjured segments was present despite a MAP of 65 mmHg, well below the autoregulatory range in normal rat spinal cord.[14]

The present experiments provide no explanation as to the mechanism by which MP affects RSCBF after injury. The dose used greatly exceeds that necessary to activate corticosteroid receptors.[3,9] During the brief 24-hour duration of these experiments, there was (not surprisingly) no evidence that MP favorably affected neurological function, which had deteriorated equivalently in both treated and untreated rats between the second and twenty-fourth hours following injury. (In the clinical trial of MP, the functional assessments that documented improvement were first performed 6 weeks following injury.)[3]

Our previous experience using a minor variation of the present experimental model

suggests that the injury is such that spontaneous improvement of function would be anticipated after approximately 4 weeks.[31] Serial histological studies by others of spinal cords thermally injured somewhat more severely than in the present experiments (time-temperature coefficient 48 °C × 60 minutes) have shown reconstitution of myelin sheaths and resolution of edema beginning at about 2 weeks after injury.[32] These histologic findings are consistent with the clinical evidence of recovery that we observed previously at 1 month.

Whether the early changes in RSCBF attributable to MP therapy documented here have functional significance in the long term is presently unknown. Early edema accumulation in the spinal cord was not altered by MP treatment in this model. These experiments provide added support for the concept that high dose MP can affect RSCBF early after cord injury. Whether this effect is direct or indirect or whether it may be merely an epiphenomenon remains to be determined.

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