Lactate and pyruvate metabolism in injured cat spinal cord before and after a single large intravenous dose of methylprednisolone

J. Mark Braughler, Ph.D., and Edward D. Hall, Ph.D.

Program in Pharmacology, Northeastern Ohio Universities, College of Medicine, Rootstown, Ohio

The lactate content and the lactate/pyruvate ratio of the acutely traumatized cat spinal cord have been studied and found to rise rapidly following a 400 gm-cm injury. Lactate levels rose nearly twofold within 5 minutes after injury, peaked at 2 hours after injury, and remained significantly elevated for at least 8 hours compared to an adjacent uninjured segment of traumatized cord. Pyruvate levels, on the other hand, fell acutely in the injured section of cord during the 1st hour after injury then rose slowly over an 8-hour period. The changes in tissue lactate and pyruvate metabolism in the spinal cord following injury are consistent with a marked injury-induced reduction in blood flow. The elevation in lactate and the fall in pyruvate levels observed at 1 hour after injury were completely prevented by the intravenous administration of a single 30-mg/kg dose of methylprednisolone sodium succinate at 30 minutes after injury. Lower or higher doses of methylprednisolone were far less effective. The effects of the 30-mg/kg dose of methylprednisolone on tissue lactate content were associated with high tissue levels of the glucocorticoid and were short-lived, paralleling the accumulation and elimination pattern of steroid from the injured tissue. The results suggest that, in addition to other reported beneficial actions of large intravenous doses (30 mg/kg) of methylprednisolone on the injured cord, the glucocorticoid may also improve blood flow to the injured segment as has been suggested by others. The use of high glucocorticoid doses, early therapy initiation, and rigorous maintenance dosing is discussed.

Key Words: methylprednisolone, spinal cord injury, lactate, pyruvate, ischemia

Ischemia is believed to play a central role in the autodestruction of spinal cord tissue following blunt trauma. It has been postulated that a reduction in the delivery of oxygen to the injured cord leads to the dissociation of normally tightly coupled electron transport chain components. Such a disruption in the normal flow of electrons to molecular oxygen may result in the production of oxygen-free radicals within the membranes of cells and organelles and the subsequent peroxidative attack of unsaturated fatty acids within those membranes. Also associated with the injury-induced ischemia is a dramatic reduction in the tissue content of high-energy phosphate compounds, as well as an elevation of lactate levels within the injured tissue.

These changes in metabolism reflect a reduction in blood flow and contribute to the overall pathological deterioration of the injured tissue and the permanent loss of sensory and motor function.

In a number of previous studies from our laboratories, we have demonstrated that single large intravenous doses of methylprednisolone sodium succinate in the 30-mg/kg range can have profound beneficial effects on spinal cord electrophysiology, (Na+ + K+)-ATPase activity, and (perhaps most importantly) on injury-induced lipid peroxidation. We have hypothesized that these high-dose glucocorticoid effects and the steroid's apparent ability at high doses to improve microvascular integrity are responsible for the demonstrated effectiveness of high-dose glucocorticoid therapy in promoting the recovery of sensory and motor function in animals following experimental spinal cord trauma. The present study was undertaken in order to define more clearly the dose-response and time action characteristics of methylprednisolone on the delivery and utilization of oxygen in the spinal cord, as assessed by its effect on lactate and pyruvate metabolism within the injured cord tissue.

Materials and Methods

Animal Preparation and Spinal Injury

Eighty-seven adult mongrel cats of either sex (each weighing 1.8 to 5.0 kg) were used in this study. The animals were prepared and the spinal cord injured.
Methylprednisolone and metabolism of injured cat spinal cord

TABLE 1

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Dose (mg/kg)</th>
<th>No. of Cats</th>
<th>Values (nmoles/mg dry weight)</th>
<th>Lactate/Pyruvate Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal untreated</td>
<td>30</td>
<td>4</td>
<td>13.3 ± 1.1</td>
<td>0.46 ± 0.05</td>
</tr>
<tr>
<td>injured untreated</td>
<td>7.5</td>
<td>4</td>
<td>34.5 ± 7.9</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>injured</td>
<td>15</td>
<td>5</td>
<td>22.4 ± 4.9</td>
<td>0.45 ± 0.15</td>
</tr>
<tr>
<td>injured</td>
<td>60</td>
<td>4</td>
<td>12.4 ± 1.2</td>
<td>0.70 ± 0.04</td>
</tr>
<tr>
<td>Methylprednisolone Assay</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In some animals, the tissue methylprednisolone content at the time of cord removal for lactate and pyruvate determinations was also measured. Frozen cord samples weighing approximately 100 mg were homogenized and extracted in a mixture of ether and methylene chloride (60:40), and assayed for methylprednisolone by high-performance liquid chromatography, as described by us in detail elsewhere.6,13

Results

A 400 gm-cm injury to the spinal cord resulted in the rapid elevation of lactate levels within the injured segment (Fig. 1 left). By 5 minutes after injury, the lactate content of the injured segment was nearly doubled compared with the control or an uninjured segment. By 2 hours, lactate levels within the injured segment were increased over threefold. Following this acute elevation, lactate levels in the injured segment declined between 4 and 8 hours after injury, but remained significantly elevated compared to the control or an uninjured segment. Lactate levels in an uninjured segment of traumatized cord did not change significantly during the 8-hour period after injury.

In contrast to lactate, tissue pyruvate content fell during the 1st hour after injury (Fig. 1 right). An increased lactate content coupled with a fall in pyruvate levels translates to a dramatic increase in the tissue lactate/pyruvate ratio (27.7 in control measurements compared with 92.4 in the injured segment at 1 hour after injury), suggesting marked ischemia. Between 2 and 8 hours after injury, the pyruvate content in the injured segment rose steadily, and at 8 hours was significantly higher than control values. It was during this period when pyruvate levels rose that lactate levels began to decline (Fig. 1 left). This observation is not unexpected since during ischemia the supply of reduced nicotinamide adenine dinucleotide (NADH) would diminish and, thus, the reducing equivalents necessary for the conversion of pyruvate to lactate by lactate dehydrogenase would be limiting. In addition, the H isozyme of lactate dehydrogenase, which may be the predominant form in most neural tissue, is strongly inhibited by its primary substrate, pyruvate. Finally, since lactate dehydrogenase contains sulphydryl groups required for activity,16 pyruvate and lactate levels may also reciprocally rise and fall, respectively, due to the direct inactivation of lactate dehydrogenase by free-radical peroxidative attack of critical enzyme sulphydryl groups. This latter speculation is supported by our recent studies demonstrating that significant free-radical reactions and lipid peroxidation occur during the 1st hour after a 400 gm-cm injury of the cat spinal cord.13 As with lactate, pyruvate levels in an uninjured segment of traumatized cord did not change appreciably during the 8-hour period after injury.

Table 1 shows the effects of the intravenous admin-

(400 gm-cm) as described previously.6,13 Briefly, animals were anesthetized with intravenous alpha-chloralose (80 mg/kg), paralyzed with intravenous gallamine triethiodide (3 mg/kg), and ventilated with room air. The spinal cord was exposed by dorsal laminectomy from L-2 to L-5. A 400 gm-cm injury was produced at the L-4 vertebral level by dropping a 50-gm weight 8 cm onto a Teflon impounder that was resting lightly on the surface of the cord with the dura mater intact. Blood pressure and somatosensory evoked potentials were continuously monitored throughout the duration of the experiment.6,13

Some animals received a single intravenous bolus injection of methylprednisolone sodium succinate (Solu-Medrol) via a brachial vein at 30 minutes after injury. The dose of methylprednisolone was varied from 7.5 to 60 mg/kg as indicated in Table 1.

Lactate and Pyruvate Assays

At various times after injury and/or drug administration, the section of traumatized cord (L-4) and a control segment (L-2) were rapidly removed, frozen in liquid nitrogen, and stored at −70°C. The time required for cord removal and freezing was less than 30 seconds. Some frozen cord sections were powdered at −70°C, and 20- to 50-mg samples of this powder were extracted in 3M perchloric acid at −10°C, neutralized with 3M KHCO3, and assayed for lactate and pyruvate content as described by Lowry, et al.18 The extracted powders were dried in tared vessels and weighted; the results are expressed as nmoles of lactate or pyruvate/mg dry tissue weight. If control lactate and pyruvate (from the uninjured cord) levels reported in this study (13.3 ± 1.1 and 0.46 ± 0.05 nmoles/mg dry weight, respectively) are converted to values based upon wet tissue weight (assuming that central nervous system (CNS) tissue is approximately 75% water by weight), the calculated wet tissue weight content of each metabolite is consistent with that reported by others for brain18 and spinal cord.1
istration of different doses of methylprednisolone 30 minutes after injury on the levels of lactate and pyruvate in the injured segment at 1 hour after injury. The injury-induced rise in lactate levels, as well as the increase in the lactate/pyruvate ratio seen at 1 hour, was completely prevented by the 30-mg/kg dose of methylprednisolone. Methylprednisolone doses of less than 30 mg/kg were less effective or were without effect, as was a higher dose of 60 mg/kg. Such a biphasic dose-response curve for methylprednisolone with an optimum dose in the 30 mg/kg range has been observed previously for its effects on spinal cord lipid peroxidation, (Na⁺ + K⁺)-ATPase activity, and motor neuron function. The ability of the 30-mg/kg methylprednisolone dose to prevent the rise in lactate formation associated with injury is probably not due to a direct effect of the steroid itself on lactic acid formation since the 30-mg/kg dose had no effect on lactate levels in the normal (uninjured) cord. These observations suggest that methylprednisolone normalized lactate levels in the injured cord through an indirect action, that is, on blood flow.

The fall in pyruvate seen at 1 hour after injury (Table 1) was prevented by all doses of methylprednisolone tested. The glucocorticoid effect to prevent or reverse the injury-induced fall in tissue pyruvate may partially represent a direct steroid effect on tissue pyruvate formation. This is indicated by the fact that the administration of methylprednisolone to animals in the absence of cord injury caused the spinal cord pyruvate content to rise significantly. The effect of methylprednisolone on pyruvate also differs from its action on lactate in that all doses tested caused an elevation of tissue pyruvate content. On the other hand, only the 30-mg/kg dose in the injured cord significantly altered lactate formation.

The time course for the effects of a 30-mg/kg dose of methylprednisolone given 30 minutes after injury on lactate levels is shown in Fig. 2, and was correlated with the levels of methylprednisolone in the injured cord segment. As demonstrated in Fig. 1 left, injury causes a rapid increase in the lactate content of the injured segment relative to the uninjured segment. The increased levels of lactate are probably related to ischemia and reflect a reduction in blood flow to the injured segment. Methylprednisolone administration 30 minutes after injury rapidly restored the lactate content of the injured segment to within normal limits (that is, there was no difference between injured and uninjured segments). This reduction in lactate levels within the injured segment occurred in conjunction with an accumulation of methylprednisolone in the injured segment which peaked at 1 hour after drug administration. These observations and the results shown in Table 1 may be interpreted as an increased blood flow and improved oxygen delivery to the injured segment caused by methylprednisolone. Beginning at 1 hour after methylprednisolone administration, the lactate content of the injured segment began to rise again, and remained elevated compared to an uninjured segment as glucocorticoid levels declined within the traumatized tissue. Lactate levels in the uninjured segment of injured spinal cord (14.3 ± 1.3 nmoles/mg dry weight before injury in eight samples) were not significantly altered by injury (13.5 ± 2.7 nmoles/mg dry weight 30 minutes after injury in four samples) or methylprednisolone administration (15.9 ± 2.3 nmoles/mg dry weight 1 hour after methylprednisolone in four samples).

Discussion

Several studies have examined spinal cord lactate and pyruvate metabolism following blunt injury as an index of blood flow and tissue ischemia. Locke, et al., provided the first biochemical evidence of posttrau-
Methylprednisolone and metabolism of injured cat spinal cord

motic cord ischemia by showing that the lactate content of injured primate spinal cord rose significantly within several minutes following trauma and remained elevated for 12 to 18 hours. In other studies from the same laboratory, Feldman, et al., demonstrated that, following circulatory arrest, spinal cord lactate levels rose as blood flow to the cord decreased. Based upon such studies, these investigators concluded that spinal cord lactate content may be a useful measure of blood flow.

In another more recent study using cats, Anderson, et al., did not observe changes in spinal cord lactate content at 2 hours post-injury, but noted that the lactate content of the injured spinal cord, as well as the lactate/pyruvate ratio, was elevated at 8 and 24 hours after injury. The reasons these investigators failed to observe altered lactate levels in injured cord at earlier times may relate to the method of cord freezing, which differs significantly from that utilized both in this study and by Locke, et al.,

Anderson, et al., froze spinal cord tissue in situ with liquid nitrogen, a procedure which required 4 to 5 minutes for complete freezing. Our procedure and that used by Locke, et al., required less than 30 seconds for cord removal and freezing. Lowry, et al., convincingly demonstrated that changes in brain glycolytic metabolites occur during ischemia; thus it is possible that freezing time may affect results and play a role in these observed differences.

In the present study, lactate levels within the injured segment of the cat spinal cord were found to increase rapidly from 5 minutes to 1 hour following trauma while pyruvate levels fell. Between 4 and 8 hours after injury, lactate levels began to decline, but remained elevated over control while pyruvate levels rose. It is clear that rapid changes in lactate production occur in the injured tissue soon after injury. Based upon the studies by Locke, et al., and Feldman, et al., the injury-induced increase in lactic acid probably reflects ischemia.

The administration of a 30-mg/kg dose of methylprednisolone at 30 minutes after injury prevented the rise in lactate content of the injured segment, suggesting an improvement in local blood flow and/or oxygen delivery. As with our earlier studies, the dose-response curve for the drug effects was biphasic, and the 30-mg/kg dose was found to be superior to either higher or lower doses of the glucocorticoid. These observations have recently been confirmed by Young and Flamm in the 400 gm-cm contused cat spinal cord. Using the hydrogen-clearance technique to estimate local blood flow, these investigators demonstrated that a 30-mg/kg

![Graph showing the correlation of the time course of methylprednisolone effects on the lactate content of injured cord with the tissue levels of steroid. The spinal cord was injured at 30 minutes before time zero. At time zero, animals received a single 30-mg/kg intravenous bolus of methylprednisolone (MP). At the times indicated, the injured and an uninjured segment of cord were removed, frozen, and later assayed for lactate content. In parallel experiments, the injured section of cord was removed and assayed for methylprednisolone content (open circles). Values represent the mean ± standard error from the number of animals indicated above each value. Lactate content is expressed as the difference between the lactate content of the injured section minus that of an uninjured section (closed circles). Significance is by Student's t-test. a = lactate content in injured cord significantly different from that of an uninjured section of injured cord (p < 0.001).](image_url)
intravenous dose of methylprednisolone given at 45 minutes after injury was far superior to a 15-mg/kg dose at increasing blood flow to the injured cord. In addition, the 30-mg/kg dose was shown to be superior in facilitating the normalization of extracellular Ca2+ concentrations and in promoting the return of somatosensory evoked potentials after injury.

In earlier studies, Anderson, et al., did not observe a decreased lactate content in the injured cat spinal cord at 2 hours after compression injury when the cords were treated with 30 mg/kg methylprednisolone at 1 hour after injury. The reasons for this may not only be related to the method of freezing, but perhaps more importantly to the times of steroid administration and cord sampling. In a recent pharmacokinetic study, we reported the uptake and elimination of a 30-mg/kg intravenous dose of methylprednisolone from the injured cord. In those experiments it was found that considerably less (one-half) steroid accumulates in the injured cord segment when drug is administered at 1 hour after injury compared to when drug is administered at 30 minutes after injury. Furthermore, it is clear from the results in Fig. 2 that the ability of methylprednisolone to improve blood flow and thus reduce the accumulation of lactate in the injured cord requires high tissue levels of glucocorticoid. This effect is also relatively short-lived, and is nearly gone within 2 hours of drug administration after tissue methylprednisolone levels have fallen substantially.

The mechanisms by which methylprednisolone may increase blood flow to the injured cord are unclear at this time. In all probability, several actions of the glucocorticoid may be involved, including: 1) a direct vasodilator action; 2) attenuation of lipid peroxidation; 3) inhibition of arachidonic acid metabolism; and 4) the alteration of alpha receptor sensitivity. Regarding the latter possibility, recent work in our laboratories has demonstrated that a single 30-mg/kg intravenous dose of methylprednisolone acts to depress the sensitivity of alpha receptors to vasoconstrictor amines in normotensive cats.

The results of the present study are consistent with the hypothesis that a reduction in blood flow occurs rapidly in the injured spinal cord tissue following blunt contusion trauma. This reduction in blood flow is evidenced by a dramatic elevation in the lactate content and lactate/pyruvate ratio within the injured tissue. The intravenous administration of a 30-mg/kg dose of methylprednisolone at 30 minutes after trauma can prevent the rise in tissue lactic acid associated with injury, perhaps through an improvement in blood flow. It is clear from this study that a dose of 30 mg/kg is required to have this beneficial action. These findings are consistent with our other observations concerning the beneficial actions of the 30-mg/kg methylprednisolone dose on lipid peroxidation, (Na+ + K+)-ATPase activity, and motor neuron function. This dose is considerably higher than those currently being used for the clinical management of CNS trauma. Furthermore, due to the relatively short-lived effect of methylprednisolone, it would appear that, in order to maintain therapeutic concentrations in the injured spinal cord, rigorous maintenance dosing is required following the earliest possible initiation of treatment.

Acknowledgments

The authors wish to thank Karen Snyder and Brigitte Hirst for their dedicated and excellent technical assistance, and the Upjohn Co., Kalamazoo, Michigan, for the generous supply of Solu-Medrol for this study.

References

5. Braughler JM, Hall ED: Pharmacokinetics of methylprednisolone in cat plasma and spinal cord following a single intravenous dose of the sodium succinate ester. Drug Metab Dispos 10:551–552, 1982
15. Hall ED, Plaster M, Braughler JM: Acute cardiovascular response to a single large intravenous dose of methylpred-


Manuscript received November 22, 1982.

Address reprint requests to: J. Mark Braughler, Ph.D., CNS Diseases Research Unit, The Upjohn Co., Kalamazoo, Michigan 49001.