Comparison of methylprednisolone with dexamethasone in treatment of acute spinal injury in rats

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Effect of methylprednisolone sodium succinate (MPSS) and its comparison with dexamethasone in experimentally induced acute spinal cord compression in adult rats was studied. The rats were divided into group A (control) and group B, which was subdivided into B1, B2, B3 where MPSS was given after 1, 8 and 24 hr and B4 where dexamethasone was given after 1 hr of cord injury respectively. Proper neurological evaluation was done with mobility, running and climbing score. Recovery index was evaluated for 7 days. After sacrificing the rats, spinal cord was observed histopathologically. Mean recovery index and microscopic findings based on hemorrhage in gray and white matter, neuronal degeneration, hematomyelia and edema in white matter were recorded. The results suggested that MPSS was effective in promoting post-traumatic clinical and histological recovery and to a greater extent, when given 1 hr after trauma. MPSS is more effective than dexamethasone in reducing edema when both are given after interval of 1 hr.

Keywords: Dexamethasone, Methylprednisolone, Rat, Spinal injury

IPC Code: Int. Cl A61 K

Acute spinal cord compression is seen in patients suffering from vertebral fractures, herniated intervertebral discs, neoplasms or hemorrhage following which a series of events take place leading to auto destruction. The main being prolonged oligemia, which produces marked depletion of high-energy phosphate reserves, lactic acidosis and tissue edema in addition to posttraumatic inflammatory response1. In experimental models of spinal cord injury, methylprednisolone has prevented posttraumatic spinal cord ischaemia especially when administered early since pathophysiological changes such as axonal and neuronal degeneration, edema and ischemia begin to occur within first 6 hr of the insult2-5. The present study was conducted to compare the efficacy of MPSS with dexamethasone in promoting clinical recovery, decreasing edema and tissue necrosis of the spinal cord following acute compression injury in rats.

Materials and Methods

Immunized and conditioned, 50 Wistar albino rats (250 to 350 g) of either sex were divided in two broad groups. Group A (control), undergoing spinal cord compression only and Group B undergoing spinal cord compression and treated with MPSS and dexamethasone. The latter was subdivided into B1, B2, and B3 where MPSS was administered after 1, 8, and 24 hr of compression respectively, and B4 where dexamethasone was administered after 1 hr of injury. Each group comprised 10 rats. Fifty rats were anaesthetized after shaving, painting and draping the lower dorsal region and three level laminectomy was performed. The dura was exposed with the intention of producing reversible injury and compressed by placing an object of 25 g weight for 2 min. Subsequently, the wound was closed meticulously in layers and sterile dressing done and the rats were placed in their respective labeled cages. One hr after injury, in 10 rats (B1) MPSS was administered at the dose of 30 mg/kg/day for the first 3 days, 15 mg/kg/day for the next 2 days, 7.5 mg/kg/day for final 2 days. In 10 rats (B4), dexamethasone was administered as 6 mg/kg/day for 3 days, 3 mg/kg/day for next 2 days, 1.5 mg/kg/day for final 2 days. MPSS was administered at the same dose and scheduled as B1 to B2 and B4 after 8 and 24 hr of injury respectively (10 rats each). These fifty rats were neurologically evaluated on a daily basis for 7-days
(recovery period). Eugene D Means & Douglas K Anderson methods was taken for the locomotor score (Table 1). The animals were sacrificed by intraperitoneal (ip) sodium pentobarbital on day 8. The spinal cord was removed after 4 hr. The injured segment of the spinal cord was identified and approximately 2 cm section was cut and fixed by immersing in cold 10% formalin. The dura was carefully removed and the spinal cord divided transversely into two pieces. Each piece was processed in routine fashion and embedded in paraffin. The tissue was serially sectioned (10 μm thick) and stained with hematoxylin and eosin. Every 10th section of the tissue was photographed on a Nikon photomicroscope using AX1 objective. Microscopically areas of hemorrhage in gray and white matter, neuronal degeneration, necrosis, edema and hematomyelia were observed. Mean recovery index and microscopic findings were compared between groups A (control) and B and between the group B1 and B4. Statistical evaluation was done. Mean values were computed as arithmetic means ±SD and compared with Chi Square test. Level of significance was set at $p<0.05$. For group A it was 5.1±1.37, group B1 was $8.2±1.23$ (*$p<0.05$, significant), B2 $6.2±2.04$, B3 $5.8±2.04$ (*$p<0.05$ not significant), and for B4 it was $6.6±1.07$ ($p<0.05$, significant).

**Results**

On calculation of the mean recovery index, it was observed that MPSS produced better clinical recovery as compared to dexamethasone, given after 1 hr following cord trauma and these results were statistically significant. It was observed in the present study that in 80% of rats (control group) the white matter edema was of moderate to severe degree, while among the steroid treated group (B1, B2, B3 and B4) it was 20, 60, 40, 60% respectively (Table 2). The histopathological evaluation also showed moderate to severe degree of gray matter hemorrhages in 70% rats of the control group, while among the steroid treated groups (B1, B2, B4, B4) it was 40, 60, 60% retrospectively (Fig. 1) and white matter hemorrhages in 60% rats of the control group, while B1, B2, B3, B4 it was 40, 60, 20% respectively (Table 3). Neuronal degeneration and necrosis as a result of ischemia was seen in 60% rats of the control group, while it was not there in B1, 40% in B2, 20% in B3 and nil in B4. But the results were statistically insignificant as $P>0.05$ in above cases with hemorrhage and degeneration (Fig. 2).

<table>
<thead>
<tr>
<th>Severity</th>
<th>Group A No. (%)</th>
<th>Group B1 No. (%)</th>
<th>Group B2 No. (%)</th>
<th>Group B3 No. (%)</th>
<th>Group B4 No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mild</td>
<td>2 (20)</td>
<td>*8 (80)</td>
<td>6 (60)</td>
<td>6 (60)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Moderate</td>
<td>8 (80)</td>
<td>2 (20)</td>
<td>4 (40)</td>
<td>4 (40)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
</tr>
</tbody>
</table>

*Significant at $P>0.05$
Discussion

The most dramatic response to spinal cord injury is the reduction in the spinal cord blood flow [SCBF]. At the level of cord injury the gray matter showed a progressive reduction in blood flow within 1-2 hr, while the changes in white matter blood flow are less dramatic\(^9\). Within 2 hr, the central petechial hemorrhages enlarge while polymorphonuclear and microglial reactions, ghost cells, eosinophilic nerve cells with indistinct nuclei, smudged cytoplasm and loss of Nissle’s bodies become evident. At 4 hr, the process advances to coagulation necrosis up to 40% of the central gray and subjacent white matter\(^10\). By 6 hr, neuronal and axonal degeneration occurs with accompanying edema, ischemia and advanced structural degeneration. Biochemical evidence of significant lipid peroxidation within the spinal cord has been demonstrated to occur within 1 hr after severe spinal cord contusion\(^11\). Thereby, one can ascertain that ischaemic hypoxia and associated oxygen derived free radical generation that lead to lipid peroxidation probably begin in the central gray matter. This would irreversibly damage myelin and axons\(^12-14\). Edema can involve gray matter also, but mainly manifests by enlargement of periaxonal spaces, swelling of axons and appearance of vacuolation in the white matter of the traumatized cord\(^15\). As seen in our finding, microscopic evaluation of white matter edema as manifested by enlargement of periaxonal spaces, swelling of axons vacuolation was of moderate to severe degree in 80% of control group while among the steroid treated group it was 20% in B1, 40% in B2, 40% in B3 and 60% in B4 respectively. At ultra structural level thickening of.

![Image](image.png)

**Fig. 1—**Gross transverse section of the spinal cord (A)—Control group A showing moderate degree of gray matter hemorrhage and white matter edema; and (B)—Treated group showing mild degree of gray matter hemorrhage and white matter edema (Group B; magnification at 25X)

**Table 3—Profile of hemorrhage in white and gray matter**

<table>
<thead>
<tr>
<th>Severity</th>
<th>Group A No. (%)</th>
<th>Group B1 No. (%)</th>
<th>Group B2 No. (%)</th>
<th>Group B3 No. (%)</th>
<th>Group B4 No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil in gray matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild in gray matter</td>
<td>3 (30)</td>
<td>6 (60)</td>
<td>4 (40)</td>
<td>4 (40)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Moderate in gray matter</td>
<td>7 (70)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>6 (60)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Total in gray matter</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Nil in white matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild in white matter</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>6 (60)</td>
<td>4 (40)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>Moderate in white matter</td>
<td>6 (60)</td>
<td>4 (40)</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Total in white matter</td>
<td>10 (100)</td>
<td>10 (100)</td>
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Value of \(p \geq 0.05\) Not significant.
capillary wall swelling of astrocyte perivascular feet and enlargement of the extra cellular and periaxonal spaces in the white matter is seen. The white matter undergoes graded changes beginning with the enlargement of the periaxonal spaces after 30 min. and attenuated myelin sheath and splaying of the myelin lamellae by 1 hr after 4 hr, 25% of the fibers manifest myelin breakdown and consequent axonal changes.16-18 These changes become maximal between 2 to 3 days after the injury and resolves within about 7 days. Thus, we can infer that to achieve significant reduction in white matter edema early institution of MPSS therapy is mandatory. This will hasten the neurological recovery by checking the pathological sequele of cord edema. A number of factors contribute to the vulnerability of the center of the cord. In contrast to the tightly packed fiber tracts of white matter, the neuropil of gray matter is easily separated by fluid or blood increase, if any, in the intramedullary pressure is concentrated centrally because of the inelastic pial membrane. Normally the gray matter : white matter blood flow ratio is 5:1. In the injured gray matter, metabolic needs may exceed the available blood flow. At the same time dorsal white matter blood flow is more severely compressed than ventral white matter blood flow. This may reflect the inherent resistance of the ventral spinal artery to compression compared with the smaller dorsal spinal arteries and pial vessels. Experimental studies indicate that the degree of involvement of any cord system is mainly a function of its distance from the center of the cord. Accordingly peripherally located fibers tend to be spared19,20. MPSS, given early, may stop this sequence of events by a direct antioxidant effect, inhibiting arachidonic acid release and thromboxane formation, increasing vascular responsiveness of to vasoactive neurotransmitters and direct vasodilatation21-23. Thus, we infer that MPSS is potent in promoting recovery post trauma especially if given in right dose and right time i.e. earliest after trauma. On comparing the neurological and histopathological observations on various groups treated with MPSS and dexamethasone, it can be inferred that MPSS is more effective than dexamethasone in promoting clinical and histological recovery and reducing edema when given earliest after trauma and these results are statistically significant.

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References


