The Effect of Prophylactic Local Epidural Steroid Delivery in a Spinal Cord Injury Model

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Abstract

Spinal cord injury during high-risk spinal deformity correction surgery occurs rarely despite best efforts to avoid it. Current neurophysiological monitoring strategies can only report an injury after it happens and some injuries fail to be captured intraoperatively. This catastrophic complication is usually irreversible. Using adult rats and an established spinal cord injury model, the objective of this study was to investigate whether prophylactic delivery of steroids directly to the region of injury via epidural injection, immediately prior to mechanical spinal cord injury, could prevent or reduce the long-term consequences of spinal cord injury.

Methods: In adult rats, a previously described model of incomplete spinal cord injury (SCI) was utilized by introducing a size 2-French embolectomy catheter through a T10 laminotomy and compressing the cord by balloon inflation for 6 minutes. There were three study groups: the treatment group with prophylactic local epidural injection of methylprednisolone (MP) 30 minutes prior to SCI (“SCI + MP”); the 1st control group with pre-operative normal saline (NS) administered epidurally 30 minutes before SCI (“SCI + NS”); and the 2nd control group with epidural injection of methylprednisolone only, without SCI (“MP only”). Rats were evaluated weekly by two blinded evaluators for a period of 6 weeks utilizing the Basso-Beattie-Bresnahan (BBB) standardized behavioral scoring system.

Results: The MP only group without SCI recovered from surgery rapidly without any behavioral indication of SCI. There was a significant improvement in average BBB scores in the MP only group at week 2-3 (versus post-operative day 1 [POD1]) in the 3-week data (p<0.0169). Significant differences in the mean BBB score were determined in the SCI+MP group at weeks 1-3 (versus POD1) in the 3-week data (p<0.0169). These behavioral improvements were not observed in the SCI+NS group over time. At 3 weeks post-SCI, both the SCI+NS and SCI+MP groups had significantly lower mean BBB scores than the MP only group (p<0.005). At 6-weeks post-SCI, mean BBB scores were significantly different between the MP only group and the SCI+NS and SCI+MP groups (p<0.05). Mean BBB scores were significantly higher for the SCI+MP group than for the SCI+NS group at weeks 2-4 (p<0.05). Six weeks after SCI, the final mean BBB scores were 11.8 for the SCI+MP group versus 0.5 for the SCI+NS group.

Conclusion: Rats treated with prophylactic, local, epidural MP prior to mechanical SCI recovered faster and to a significantly greater extent compared to those treated with saline only. Furthermore, epidural MP administration without SCI did not have any appreciable negative effects. Prophylactic treatment of high-risk spinal deformity surgery patients with a high concentration of epidural or intrathecal methylprednisolone may have potential to mitigate SCI severity. This possibility deserves further investigation in animals and human subjects.

INTRODUCTION

Acute spinal cord injury (SCI) is often a debilitating event with repercussions that may persist for the rest of a patient’s life. The incidence of SCI in the setting of spine surgery has increased with the introduction of new instrumentation and complex surgical procedures. A recent review found the incidence of intraoperative neuromonitoring changes to be 9.8% in high risk patients [1].

Prior studies have identified pre-operative risk factors for neurological complication in patients with spinal deformities. These include hyperkyphosis, neuromuscular scoliosis, congenital scoliosis, Cobb’s angle greater than 90 degrees, and pre-existing neurological impairment [2]. Surgical risk factors include combined anterior/posterior approach, harvesting of segmental vessels, significant curve correction, use of sublaminar wires, and revision surgery [3-5].

During spine surgery, acute SCI usually results from contusions or compressions of the spinal cord rather than physical transections. The primary lesion is gradually enlarged by delayed secondary damaging processes, such as lipid peroxidation, which may begin minutes after SCI and continue for weeks [6,7]. Such processes may ultimately result in loss of motor and sensory function. The specific mechanism by which secondary damage occurs is not fully understood, yet neuroprotective pharmacologic agents demonstrate the potential to prevent or reduce the resulting neurological impairment [7-9].

The intravenous (i.v.) administration of corticosteroids, specifically the corticosteroid methylprednisolone (MP), is the
current standard for treating SCI [9-14]. Nevertheless, systemic MP treatment is associated with a number of side effects that include increased risk of gastrointestinal and pulmonary complications, infections, and pneumonia [10,11,15-18]. These side effects and the relative lack of efficacy of i.v. MP [7,14,17,19], make the risk-benefit ratio unfavorable.

It is apparent that the timing of treatment and the site of administration are two critical issues regarding steroid treatment of SCI. As secondary damaging processes may begin within 5 minutes after SCI [6,7], there is little time for discovery and effective treatment before secondary injury mechanisms are set in progress. Although current methods highlight the importance of immediate i.v. administration of steroids after SCI, the emphasis on treating spinal cord trauma at the earliest possible time supports the hypothesis that a prophylactic, local injection of MP may provide maximal and immediate steroid effect while eliminating the potential for systemic complications.

Using adult rats and an established SCI model, this study aimed to investigate whether a prophylactic injection of the local epidural space with MP prior to mechanical SCI can effectively reduce the long-term consequences of SCI. Furthermore, this study was performed to investigate whether high-dose epidural MP treatment alone is associated with adverse effects on rat health and spinal cord function.

METHODS

Thirty-five female Sprague-Dawley rats (mean age: 75-85 days; mean weight: 225-250 g; Charles River Laboratories, Wilmington, MA) were used in this study. The experiments were approved in accordance with the rules and regulations set by the Institutional Animal Care and Use Committee (IACUC) at New York University Langone Medical Center. Animals were housed in an environment with controlled light/dark cycles and allowed to acclimate to their surroundings for at least one week prior to the study initiation.

Study Groups

Rat were divided into the following three groups: (1) the SCI + Saline control group ("SCI+NS", N = 11), in which an epidural injection of 10 µL normal saline was administered 30 minutes before SCI; (2) the SCI+ methylprednisolone experimental group ("SCI+MP", N = 17), in which an epidural injection of 625 µg of methylprednisolone in 10 µL of saline was administered 30 minutes before balloon inflation; and (3), the "MP only" control group ("MP only", N = 7), in which 625 µg of methylprednisolone in 10 µL of saline was administered epidurally without balloon inflation. This dosage represented the maximum amount of MP which would dissolve into 10 µL of saline.

Surgery

Induction was initiated with 4% isoflurane exposure in a chamber followed by intraperitoneal (I.P.) injection of ketamine and xylazine (80 mg/kg and 5 mg/kg, respectively). The surgical site was shaved and sterilized and the depth of anesthesia was assessed by response to tail, pinnae, or pedal pinch.

Epidural Injection

A 2 cm skin and fascial incision was made from T7 to T11 and the paraspinal musculature was excised and retracted to expose the T9-T10 vertebral levels. Part of the rostral T10 spinous process was excised with a bone cutter to improve spinal canal access. A small laminotomy was made at the caudal edge of the T9 vertebral lamina and a 33-gauge blunt needle (Hamilton, 7762-06) connected to a 10 µL glass micro syringe (Hamilton, 7635-01) was used to inject a volume of 10 µL of solution (625 µg MP in 10 µL NS or vehicle only) into the epidural space. The blunt needle was advanced from the T9-T10 interspace to approximately T8. After epidural injection was performed, 30 minutes were allowed to pass prior to balloon SCI. The animal remained under anesthesia continuously until completion of the SCI procedure.

Spinal Cord Injury and Closure

Thirty minutes after the epidural injection, a 2-French Embolecomy Catheter (120602F, Edwards Lifesciences) was inserted into the epidural space and advanced cranially such that the center of the balloon rested at the level of the spinal cord corresponding to the T8-T9 vertebral level – a method previously described by Saganova et al [20]. The balloon was then inflated to its maximal capacity (50 µL) and held constant for 6 minutes, after which the balloon was deflated and the catheter was removed. Soft tissues and skin layers were sutured and a subcutaneous injection of 0.12 mg/kg buprenorphine was administered while the rat was still sedated.

Postoperative Care

After surgery, the rats were given 0.12 mg/kg buprenorphine subcutaneously twice a day at postoperative days 1-3. Rats were housed 1 per cage with food and water ad libitum. Trans Gel packs (Charles River Laboratories) were used as a food source immediately after surgery while incomplete paraplegia was evident. Manual bladder expression via palpation was performed twice daily until the return of bladder function.

Behavioral Analysis of Rat Recovery

The locomotor performance of each animal was analyzed using the Basso-Beattie-Bresnahan (BBB) open-field scoring system [21] by two blinded observers. The BBB 21-point scale provides a gross indication of locomotor ability and determines the phases of locomotor recovery. Scoring was performed in an open area. Individual hindlimb scores were averaged between observers in order to obtain one mean BBB score per rat per week.

Histology and Histomorphometry

Histology was performed on 5 animals from the SCI+MP group, 4 in the SCI+NS group, and 4 in the MP only group at a mean time point of 8.43 weeks after SCI. Animals were euthanized with an overdose of sodium pentobarbital and perfused transcardially with 250ml of pH 7.4 phosphate-buffered saline followed by 250ml of 4% paraformaldehyde. A segment extending 1 cm on either side of the lesion was dissected from each spinal cord, post-fixed in 4% paraformaldehyde, and cryoprotected overnight in a 30% sucrose solution. The spinal cords were embedded in paraffin, cut in 5 µm serial sections, and stained with Luxol fast blue. Within each lesion, the three sections identified as the "most damaged" by a certified neuropathologist blinded to the
treatment groups were used for histomorphometric analysis of the extent of SCI. Damaged and overall areas were identified and defined by the pathologist. Morphometric lesion area quantification was performed with Surgimap software (Version 1.1.2.270). Statistical analysis was performed using one-way Analysis of Variance (ANOVA). Data represent the mean percent survival values of all animals per group ± SEM.

**Statistical Analysis**

Differences between treatment groups with respect to BBB scores were analyzed by means of a Kruskall-Wallis H Test and Mann-Whitney U Test. These statistical tests were chosen due to the small, unequal sample sizes in each group. Bonferroni Correction was used to preserve the type I error to be no more than 5% standard.

Paired T-tests with Sidak’s correction were used to analyze within-group differences over time. For the within-group analysis, a p-value of ≤ 0.0169 (one-sided) was considered significant for the 3-week functional recovery data. A p-value of ≤ 0.0085 was considered significant for the 6-week functional recovery data. Data represent the mean BBB values of all the animals per group ± SEM. Independent analyses at 3 and 6 weeks were performed because group sizes were dynamic due to greater mortality in the saline-treated SCI animals.

**RESULTS**

Due to significant mortality, which was greater in the saline-treated group, the sample sizes were different at the study conclusion than at initiation. Therefore, statistical analysis was performed for the study midpoint and at conclusion.

**Functional recovery 3 weeks after SCI**

The epidural administration of MP before SCI resulted in a significant recovery between post-operative day 1 (POD1) and weeks 1-3 for the SCI+MP rats (N=11) (p<0.0169). A steady and significant rise in the mean BBB scores from POD1 (BBB = 1.5) through weeks 1-3 (BBB = 3.8, 6.6, 7.5; p<0.0169) was also observed (Figure 1). In the MP only group (N=7), there was also a significant recovery between POD1 and weeks 2-3 (p<0.0169). The mean POD1 BBB score for the SCI+NS rats (N=7) was 0.1 (out of 21). Although the mean BBB score for this group rose to BBB=1.3 at 3 weeks after SCI, there was no significant recovery between POD1 and 1-3 weeks (Figure 2).

In the 3 week analysis, there was no significant difference in mean BBB scores of the SCI+MP group compared to the SCI+NS group at any timepoint (POD1 to Week 3).

**Functional recovery 6 weeks after SCI**

The SCI+MP group exhibited significantly higher scores than the SCI+NS group at weeks 2-4 in the 6 week analysis of behavioral recovery. Two weeks after SCI, the SCI+MP group had recovered to BBB=8.8 whereas the SCI+NS group had recovered to BBB=0.2 (p<0.05). At 3 weeks after SCI, the SCI+MP group had gained another 1.2 points to reach BBB=10 whereas the SCI+NS group remained at BBB=0.2 (p<0.05). At 4 weeks after SCI, SCI+MP and SCI+NS group scores were 10.4 and 0.7, respectively (p<0.05). There was no significant difference between each group at POD1 and Weeks 1, 5, and 6. There were no significant within-group differences for any group in the 6 week analysis (p>0.0085).

Within the first 3-weeks after SCI there was a high mortality rate in the SCI groups: 6/17 (35%) and 4/11 (36%) from the SCI+MP and SCI+NS groups, respectively. No animals from the MP only group died prematurely. Between 3- and 6-weeks after SCI, one additional animal in the SCI+MP group died and 2 from the SCI+NS group for an overall mortality of 41% and 54% for the SCI+MP and SCI+NS groups, respectively.
Quantification of Damaged Area in Lesion after SCI

Based on histological examination by a blinded neuropathologist, injured tissue area was quantified (Figure 3). Mean percent survival of spinal cord tissue at the lesion site was 49.5 ± 21%, 5.6 ± 3%, and 97.4 ± 2% for the SCI+MP (n=5), SCI+NS (n=4), and MP only (n=4) groups, respectively (p<0.05) (Figure 4). Significant differences between groups were only demonstrated between the SCI+NS and MP only groups in post-hoc analysis.

DISCUSSION

Intraoperative spinal cord injury (SCI) resulting in persistent neurological deficit is a rare but exceedingly serious complication associated with complex spinal deformity correction surgery. Post-operative neurological deficits vary in both functional and temporal severity and may range from minor deficits to paraplegia or even quadriplegia. These deficits may result from mechanical trauma, vascular injury, and/or metabolic insults to the spinal cord during surgery.

Various pharmacological therapies have been used or developed in the attempt to treat SCI, including corticosteroids [6,11,22,23], gangliosides [24-27], opioid antagonists [10,28-31], glutamate receptor and ion channel antagonists [32-36], and cyclooxygenase inhibitors [37-39]. Of these therapies, corticosteroids have been most used clinically because of their availability and familiarity, and several studies have demonstrated their efficacy in helping both human and animal subjects recover from SCI [7-9,19,23].

Results of the Second National Acute Spinal Cord Injury Study (NASCIS II) have shown that intravenous (i.v.) methylprednisolone (MP) treatment improves neurologic outcome in spinal cord-injured patients when administered in a 24 hour intensive high dose regimen beginning within 8 hours of the SCI [10,11,23]. However, systemic MP treatment has also been associated with a higher rate of complications, including severe pneumonia, sepsis, and pulmonary and gastrointestinal complications [14-18,40-44]. Furthermore, i.v. administration of MP is also associated with inadequate efficacy [15,17,18,40-45]. Therefore, there is need for a local, targeted, and immediate approach for the mitigation of SCI. The present study evaluated whether the prophylactic, epidural injection of a single high-concentration MP bolus at the site of spinal cord compression would efficiently reduce adverse observable effects of SCI.

The balloon inflation technique and duration of compression utilized in the present study caused a severe SCI, as indicated by the consistently low post-operative BBB scores, particularly in the saline treated group. Despite this severe injury, the 6 week post-injury recovery analysis demonstrated that a single prophylactic, high-dose bolus of MP, injected epidurally prior to induction of mechanical SCI, resulted in significantly greater recovery - compared to the SCI+NS group - in rats at weeks 2-4 after SCI to a BBB score that indicates ability to ambulate. Overall, we observed 6- and 11-point average functional improvements at 3- and 6-weeks after SCI, respectively; in contrast, studies providing i.v. MP after SCI in the rat observed roughly 3-point improvements at 4 weeks after SCI [46,47].

The successful recovery of the MP only group - indicated by high BBB scores and small score variability between rats - demonstrated that local high-dose epidural steroid administration in the absence of SCI did not produce an adverse outcome.
Our histology results correspond to the observed behavioral recovery: the SCI+MP group had, on average, a 49% spinal cord tissue survival rate compared to a 56% rate for the SCI+NS group. However, this difference did not reach statistical significance is likely attributable to the small sample population per treatment group.

Our results confirm the growing body of evidence in the literature that lends support for a local approach when treating SCI. By administering a high-dose epidural bolus prophylactically, it may be possible to offer a degree of neuroprotection against the secondary tissue damage that follows mechanical contusion to the spinal cord. This approach may improve efficacy while reducing the absolute amount of total steroid delivered, thus avoiding a lengthy systemic dosing regimen that may be associated with significant side effects [48,49], and complications such as sepsis, wound infection, and pneumonia [14-16,18,40-44]. Furthermore, by using one high dose epidural bolus, the surgeon may eliminate the need for more complicated procedures that provide prophylactic steroids, such as intrathecal (IT) catheter placement or attachment of nanoparticles to the dura mater [50].

Although we ultimately performed an epidural injection of MP, consideration was given to an IT injection before SCI. A prophylactic IT injection would provide the added benefit of a more direct steroid effect on spinal cord tissue, and is a critical future topic to explore. However, an epidural injection may remain advantageous due to its relative ease of access. There is evidence that epidural corticosteroids may have a rapid transport route across the dura to the axons of the spinal cord [51]. Furthermore, transdural diffusion of MP may be significantly enhanced after dural injury, as demonstrated by spinal cord tissue diffusion measurements after epidural placement of MP-nanoparticles 5 minutes after SCI [50].

This presents a potential advantage to an epidural steroid approach by demonstrating that pre-treatment of the epidural space with steroids would enable MP diffusion only if appreciable damage occurred to the dura mater – indicative of acute mechanical SCI. Nevertheless, compression of the spinal cord can occur without visible damage to the dura mater, and exploration of prophylactic IT steroid treatment remains of interest.

STUDY LIMITATIONS

There was wide variability in rat locomotive function in the SCI+MP group. This result may potentially be attributed to three causes that could hypothetically influence lesion severity: (a) black-flow of steroid solution out of the epidural space during prophylactic administration; (b) partial balloon deflation in the epidural space due to the physical constraints inside the vertebral canal; and (c) discrepant damage to the dura mater resulting in differential transdural diffusion of steroids. Furthermore, we were unable to use all animals for the histomorphometric analysis. This portion of the study was therefore underpowered to show a statistical difference.

CONCLUSION

The use of high-dose epidural methylprednisolone 30 min prior to severe, mechanically-induced spinal cord injury using balloon inflation was associated with significantly improved behavioral recovery compared to normal saline control. Compared to literature reports of systemic corticosteroids, epidural administration may have distinct advantages with potential for clinical application in the case of spinal cord injury. At this time, more experiments are needed to confirm that the administration of local steroids is associated with improved locomotive recovery as well as a decreased risk of systemic and neurologic complications. Attention should also be given to explore the use of epidural steroids after SCI as well as the intrathecal administration of steroids both before and after SCI in an animal model.

REFERENCES

