Protective effect of dorsal longitudinal myelotomy at 72 Hours after spinal cord injury in rat model

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Abstract

Incision of the spinal cord (myelotomy) after spinal cord injury (SCI) is a surgical treatment which is mainly performed within 24 hours after SCI. However, many patients are first seen outside the established time window of 24 hours. Furthermore, little attention has been given to its efficacy and mechanism after 24 hours. In this study, dorsal longitudinal incision of spinal cord on SCI rats was performed at 72 hours after SCI to remove the liquefied necrotic gray matter. The results indicated that after myelotomy, locomotor function of hindlimbs significantly improved from 21st day after SCI. There was also increased residual white matter area at the rostral and caudal segments to the injury center when examined at day 42 after the injury. These results suggested that myelotomy at 72 hours after injury has protective effect on the spinal cord.

INTRODUCTION

Spinal cord injury (SCI) is a severe illness of central nerve system (CNS) which results in huge economic and psychological burden on society and family.1,2 Because of the growth and differentiation specificity of the CNS cells and the release of a series of injury factors and antibodies, it is difficult for mammals to regenerate CNS cells after injury.3 After SCI, hemorrhage in gray matter occurs immediately which results in the degeneration and necrosis of CNS cells at primary stage. This is followed by secondary damage which includes inflammatory factors release, mitochondrial damage, energy supply reduction, apoptosis, and necrosis. All these events lead to occurrence of Wallerian degeneration and loss of nerve conduction and subsequently increases the limb dysfunction.4,5 About a century ago, Alfred R. Allen developed spinal cord contusion experiment in animals. He also operated on patients who suffered SCI.6 Several experiments suggested that medial longitudinal myelotomy was structurally and functionally beneficial for SCI. Based on the understanding of this pathological process, spinal cord decompression is performed within 24 hours after SCI to release the acutely accumulated fluid in spinal cord, to reduce the tension on the nerve tissue with beneficial therapeutic effect.7,10 The purpose of myelotomy after the exudative phase of acute bleeding is to clear liquefaction necrosis in gray matter and improve the internal environment of spinal cord.11,12 However, many patients are first seen outside the established time window of 24 hours. Till to date, little attention was given to its efficacy and mechanism more than 24 hours after the SCI. In this study, we investigated whether the spinal cord dorsal incision and removal of the liquefaction necrosis in gray matter at 72 hours after SCI would affect the neurological outcome in rat, and studied the effect of the surgery on the spinal cord.

METHODS

Animals and groupings

Forty five adult male Sprague-Dawley rats weighing 250–300 g were studied. The experimental protocol was approved by the Capital University of Medical Science Institutional Animal Care and Use Committee. The animals were classified into 3 groups. Group A, sham group: laminectomy without contusion; Group B, control group: laminectomy with contusion, after 72 hours spinal cord exposure without myelotomy; Group C, myelotomy group: laminectomy with contusion, myelotomy and debridement 72 hours after contusion.

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Spinal cord injury and myelotomy

Rats were anesthetized with intraperitoneal injection of pentobarbital (50mg/kg). Cefazolin (50mg/kg) was injected subcutaneously before surgery. The surgical site was shaved and swabbed with alcohol. A midline skin incision was made to expose T8-T12 spinal column. After stripping the paraspinal muscles, laminectomy was performed at T10 to expose the spinal cord. The exposure area was about 2.5-3mm². Contusion injury was performed with the NYU (New York University) weight drop device. The crackdown was 25g•cm to result in moderate contusion and intact dura. The wound was then sutured layer by layer. Urinary bladders were emptied by manual pressure every 8 hours until recovery or sacrifice.

Seventy two hours after SCI, rats in Groups B and C were anesthetized again. In Group B (control group), the original incision was reopened and the spinal cord was exposed, but no myelotomy was performed. The animals in Group C were operated under microscope along the original incision which was opened layer by layer. The granulation tissue, which covered the surface of the spinal cord was removed. Under microscope, the dura mater was found intact, under tension and poorly pulsatile. Through the dura mater old dark brown hemorrhage could be seen under the subarachnoid space. According to our previous experience and published data, we used microsyringe needle to puncture a small hole on the dura mater, slightly away from the middle line in order to avoid the dorsal vessel. The dura mater was cut with microscissors, arachnoid was separated, spinal cord was incised partially and longitudinally along the posterolateral sulcus at depth of about 1-1.5mm. During the procedure, some dust-color or reddish-brown material consisted of old blood, cerebrospinal fluid and necrosis-like materials flowed out of the operation area. The local area and operative cavity was washed gently twice using saline. The dura mater was closed, a gelatin sponge (2×2mm) was placed on the surface of the dura mater and the muscle and skin were sutured.

Histopathology examination

Forty two days after SCI, the rats were perfused transcardially with 37°C 0.9% saline and subsequently with 500 ml 4% paraformaldehyde in 0.2 M phosphate buffer solution (pH 7.4), under deep anesthesia. The spinal cord including 1.5cm from the head to the end of the injury areas was quickly separated and removed and was fixed in same fixative at 4°C, and was cut into three blocks (the injury center, rostral and caudal segments). The rostral and caudal penumbras were 2.5-5.0 mm from the injury center. Paraffin-embedded specimen were continuously sliced at 4 µm and put on gelatin coated slides, for hematoxylin and eosin (HE) staining and luxol fast blue (LFB) staining of myelin. Briefly for LFB staining, the sections were treated with 0.1% LFB solution (Sigma Chemical, St Louis, MO, USA) with 95% ethanol at 60°C overnight, then with 0.01% Li2CO3 for color separation. The staining was observed under microscope (Olympus, BX51, Japan) and analyzed with software Image-Pro Plus 6.0 to calculate the proportion of residual white matter area (= residual white matter area/total white matter area×100). The image processing was interactively operated with Image-Pro plus 6.0 (IPP) to determine the various thresholds to delineate these areas.

Statistic analysis

Statistic analysis was performed using SPSS 13.0. Mann-Whitney U test was used for BBB scores and for the percentage of spared white matter area. All values were given as mean±SEM, p < 0.05 was set as significant level.

RESULTS

Locomotor function

The scores of the (BBB) locomotor rating scale of all rats were 21 before contusion. Group A continued to score 21 during the whole observation period. For Groups B and C, all rats showed complete bilateral hindlimb paralysis at the first day after SCI (Figure 1). There was no observable hindlimb movement or only slight movement of one or two joints at the first 2-4 days after injury. Group C animals also showed reluctance in activities and poor feeding. After operation, animals in Group C showed a higher BBB score than animals in Group B from 6 days after SCI and reached significant difference from day 21 (13.14±3.32 in Group C vs 10.10±0.89 in Group B).

Behavior test

We used the Basso-Beattie-Bresnahan (BBB) locomotor rating scale to evaluate rat limbs motion function. The rats were examined daily for one week, then at day 14, day 21, day 28, day 35 and day 42. Locomotion was analyzed according to the 21 point BBB rating scale. Two observers made the evaluation independently.
in Group B, \( p = 0.044 \) till the end of the study \( (p = 0.002, 0.002, 0.016, \) at day 28, 35 and 42 respectively). In addition, rats in Group C (mean BBB scores, 13.14±3.32) had frequent to consistent weight-support planter steps and frequent FL-HL coordination according to the open field test. Although animals in Group B also regained some motor functions, the BBB score was lower and only demonstrated occasional weight-support planter steps but no FL-HL coordination. At day 28 after SCI (15.08±2.52 in Group C, 11.20±1.30 in Group B, \( p = 0.002 < 0.05 \)), At day 35 after SCI (16.75±2.05 in Group C, 11.50±1.00 in Group B, \( p = 0.002 < 0.05 \)), At day 42 after SCI (18.06±1.61 in Group C, 14.37±1.89 in Group B), \( p = 0.016 < 0.05 \).

**Histopathology**

Figure 2 shows the morphology of the spinal cord slices with LFB stain at day 42 after SCI. As shown, in Group A, the spinal cord remained intact (Figure 2 a-c). However, in Group B (Figure 2 d-f) and Group C (Figure 2 g-i), white matter demyelination was easily seen. When compared with Group B, Group C had less demyelination with more retained white matter at the rostral and caudal segments. In the injury center, there was little residual white matter retained and small cystic formation (Figure 2, hollow arrow). The central gray matter showed cavities from necrosis (Figure 2, solid arrow).

The estimated proportion of residual white matter area relative to the whole white matter area at day 42 after injury of the three groups was shown in Figure 3. In Group A, the proportion of spared white matter was the same at the center, rostral and caudal segments. In Group B and C, the proportion of residual white matter area was higher at the rostral and caudal segments as compared to the center segment. The proportion of residual white matter area at the rostral penumbra of Group C (48.89±12.15%) was significantly larger than the same area in Group B (28.69±9.03%, \( p = 0.01 \)). The proportion of residual white matter area at the caudal penumbra of Group C (55.94±15.82%) was also significantly larger than the same area of Group B (21.31±9.18%, \( p = 0.020 \)). In the injury center however, there was no significant difference in the residual white matter between Group B and Group C (25.40±7.62% vs 30.64±7.88%, \( p = 0.256 \)).

**DISCUSSION**

Myelotomy has a long history for treatment of various spinal cord illnesses, such as in spinal spasticity, spinal cord tumor excision, and spinal cord decompression at acute stage.17,18 Dorsal longitudinal myelotomy has been used
Figure 2. Luxol fast blue (LFB) staining of spinal cord at rostral, center and caudal segments 42 days after SCI in Groups A, B and C. Demyelination can be seen at the white matter in the periphery of spinal cord at rostral and caudal segments. There are more tissue damage with cysts formation in the center segment.

Figure 3. The residual white matter areas (spared white matter area/total white matter area×100) in the three study groups at day 42 after SCI. Significantly larger white matter area is preserved in Group C as compared to Group B at both the rostral and caudal segments (p<0.05). Data represent means±SEM.
to decompress the spinal cord in SCI, usually at acute phase during the first 24 hours.\textsuperscript{10,14,19}

This study investigated the effect of myelotomy 72 hours after SCI. This is based on the following considerations. Firstly, within 24 hours after injury, the nervous tissue has yet to complete the liquefaction and necrosis process. It is more difficult to completely remove the hemorrhage and necrotic materials, thus affecting the efficacy of myelotomy. Secondly, as time progress, the spot hemorrhage enlarged into larger hemorrhage. By three days after injury, gray matter has evolved into liquefaction and necrosis. At 72 hours, oligodendrocytes apoptosis increased and spread to more white matter.\textsuperscript{20,21} By clearing the liquefaction and necrosis materials, myelotomy performed at this stage may alter the microenvironment thereby reducing the degeneration of white matter and preserving more nerve conduction function. Thirdly, at the first 24 hours after SCI, especially the first 8 hours is the most opportune time for interventional management.\textsuperscript{22–24} However, most patients, especially those in developing countries were not sent to the appropriate treatment centers within 24 hours, which resulted in missed opportunities for surgical intervention. Besides, in some patients who underwent surgical decompression and bone fixation, almost no neurological rescue measures were undertaken. In addition, a few case reports showed the efficacy of myelotomy performed after 24 hours following SCI. However, there is to-date limited evidence for benefits from delayed myelotomy, thus the need for this study.

Our study showed that myelotomy at 72 hours is helpful for rat after SCI. At day 42 after SCI, when compared with control group, the rats with myelotomy had milder demyelination and more residual white matter in both ends (rostral and caudal) to the injury center. In the injury center, both the myelotomy group and control group showed obvious demyelination, indicating that the myelotomy had little protective effect on the center of injury. In assessing the functional outcome using BBB rating scale, the recovery after injury can be divided into three phases, early, intermediate and late transition.\textsuperscript{15} In our study, there was no difference in the BBB score between the myelopathy and control groups during the early phase, when all rats had paraparesis and were dragging the hind legs. From day 21 after SCI, the myelotomy group scored higher than the control. This superiority persisted significantly to the end of the observation period. Rats showed plantar foot and sustained coordination of fore and hind limbs movement.\textsuperscript{15} From the day 28, while myelotomy group achieved scores of 14 to 21, the control group still had occasional to frequent foot walking and poor coordination of fore and hind limbs movement. Myelotomy at 72 hours is thus helpful for the recovery of rats’ hindlimb motor function. The benefits of myelotomy could be seen from day 21 after injury.

Based on the concept of primary and secondary injury of SCI, more and more research are focusing on prevention of secondary damage, such as inhibiting cell apoptosis, use of nerve nutrition drugs and stem cells.\textsuperscript{25} So far it is not possible to completely reverse secondary injury\textsuperscript{26}, myelotomy related techniques should thus be considered. Myelotomy may prevent secondary damage by removal of necrotic material and various negative factors in the injury tissues. The liquefied necrotic materials in the injured spinal cord may be analogous to boil or abscess, where incision and drainage has proven to be an effective therapeutic stategy.\textsuperscript{27}

Although the time course of development after injury in rat and human is not identical, we believe that this study showing beneficial effect of myelotomy at 72 hours after SCI in rats also has some clinical implications for human. The white matter edema was reported to peak by 48–72 hours after injury in moderate contusions in rats.\textsuperscript{28} In human, although there was no detailed study, many clinical studies have observed oedema progressing early after injury and by 72 hours.\textsuperscript{29,30} As in rats, the nerve fibers inside the dura matter in human may be compromised further by increasing oedema in the injury tissues. Any surgery to release this tension while oedema is still important may contribute to eventual functional recovery.

In conclusion, this study affirms the efficacy of myelotomy at 72 hours after SCI in rat. Further studies are required to elucidate the exact mechanisms contributing to the beneficial effects. There is also a need to explore the role of myelotomy in SCI beyond the 72 hours window period.

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