Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Spinal cord decompression reduces rat neural cell apoptosis secondary to spinal cord injury*

Kan XU[†1], Qi-xin CHEN[1], Fang-cai LI[1], Wei-shan CHEN[1], Min LIN[2], Qiong-hua WU[1]

([1]Department of Orthopaedic Surgery; [2]Department of Pathology, the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, China)

[†]E-mail: xukan@medmail.com.cn

Received May 13, 2008; Revision accepted Sept. 23, 2008; Crosschecked Jan. 20, 2009

Abstract: Objective: To determine whether spinal cord decompression plays a role in neural cell apoptosis after spinal cord injury. Study design: We used an animal model of compressive spinal cord injury with incomplete paraparesis to evaluate neural cell apoptosis after decompression. Apoptosis and cellular damage were assessed by staining with terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) and immunostaining for caspase-3, Bcl-2 and Bax. Methods: Experiments were conducted in male Sprague-Dawley rats (n=78) weighing 300~400 g. The spinal cord was compressed posteriorly at T10 level using a custom-made screw for 6 h, 24 h or continuously, followed by decompression by removal of the screw. The rats were sacrificed on Day 1 or 3 or in Week 1 or 4 post-decompression. The spinal cord was removed en bloc and examined at lesion site, rostral site and caudal site (7.5 mm away from the lesion). Results: The numbers of TUNEL-positive cells were significantly lower at the site of decompression on Day 1, and also at the rostral and caudal sites between Day 3 and Week 4 post-decompression, compared with the persistently compressed group. The numbers of cells between Day 1 and Week 4 were immunoreactive to caspase-3 and B-cell lymphoma-2 (Bcl-2)-associated X-protein (Bax), but not to Bcl-2, correlated with those of TUNEL-positive cells. Conclusion: Our results suggest that decompression reduces neural cell apoptosis following spinal cord injury.

Key words: Spinal cord injury, Decompression, Apoptosis, Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate nick-end labelling (TUNEL), Caspase-3, B-cell lymphoma-2 (Bcl-2), Bcl-2-associated X-protein (Bax)
doi:10.1631/jzus.B0820161 **Document code:** A **CLC number:** R36

INTRODUCTION

Recent reports have shown that apoptosis occurring secondary to spinal cord injury (SCI) contributes to further neural damage and functional loss following spinal cord trauma (Crowe *et al*., 1997; Shuman *et al*., 1997; Emery *et al*., 1998). Apoptosis is determined by a genetic program, dependent on active protein synthesis, and characterized by nuclear fragmentation and appearance of apoptotic bodies seen as small basophilic material within the nucleus or as basophilic material extruded from the cell within the blebs of the cytoplasm (Liu *et al*., 1997). The neurological status after spinal cord trauma primarily depends on the extent of neural element loss and function of the residual neural tissue. Hence, it is important to reduce any secondary neural damage, including apoptosis, which occurs after the initial traumatic SCI. Several experimental studies have suggested that decrement of neural cell apoptosis plays an important role in the neurological recovery process following incomplete SCI (Wada *et al*., 1999; Springer *et al*., 1999; Saito *et al*., 2000).

There is compelling evidence from laboratory studies in animal models that persistent compression of the spinal cord is a potentially reversible form of secondary injury. The severity of SCI in animal

* Project (No. Y207216) supported by the Natural Science Foundation of Zhejiang Province, China

models is related to the force and duration of compression, displacement, impulse, and kinetic energy (Dolan *et al*., 1980; Guha *et al*., 1987; Panjabi, 1987; Turker *et al*., 2005; Kasahara *et al*., 2006). Numerous experimental studies of decompression after SCI have been performed in various animal models (Aki and Toya, 1984; Nyström and Berglund, 1988; Delamarter *et al*., 1995; Carlson *et al*., 1997; Baydin *et al*., 2007). These studies have consistently shown that neurologic recovery is enhanced by early decompression, while there are few reports about the role of the decompression concerning with the dynamic change of the apoptosis after SCI.

Dimar *et al*.(1999) used the New York University (NYU) weight drop model to produce thoracic SCI in rats and an epidural spacer placed adjacent to the contusion to mimic the effect of persisting compression. Neurologic recovery was significantly dependent on time to decompression, with significant differences seen in all experimental groups. This study provides the strongest experimental evidence to date of a clear beneficial effect of spinal cord decompression after SCI. It is not so easy to control the spacer in the right position above the dura matter. So, another new mechanical compressive model should be established in order to observe the histopathology after the spinal cord decompression.

To investigate the neural cell apoptosis after decompression, we extended these studies by examining the histopathological effect of spinal cord compression and decompression in a rat model. Thus, in the present experimental study, we used a rat SCI model with acute spinal cord compression/decompression to investigate the dynamic changes in the populations of terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP) nick-end labelling (TUNEL)-, caspase-3-, B-cell lymphoma-2 (Bcl-2)- and Bcl-2-associated X-protein (Bax)-positive cells, representing apoptotic cells, within the spinal cord after compression/decompression.

MATERIALS AND METHODS

Experimental animals and surgical preparation

The experimental study was conducted in 78 Sprague-Dawley rats weighing 300~400 g [(350±30) g]. Following anaesthesia by an intraperitoneal injection of sodium pentobarbiturate (0.05 mg/g body weight), the rats were fixed prone on a surgical table. A limited laminectomy was performed at T10 level to expose the dura mater and the spinal cord, using a microairtome under a surgical microscope without damaging the spinal cord. A custom-made mini-screw (2 mm in diameter, 2 mm long, with a flat and smooth screw tip) was screwed slowly and vertically on the midline spinal cord for 1-mm depth through the dura mater (Fig.1a). The rats were then divided into three subgroups: Group A (n=24), decompression at 6 h after compression; Group B (n=24), decompression at 24 h after compression; Group C (n=24), persistent compression. Following the compression, the surgical wound was closed. In Groups A and B, 6 and 24 h later, respectively, the rats were re-anaesthetised to remove the screw inserted into the spinal cord. The wound was sutured in layers, while in rats of Group C, the screw was kept on the dura mater for persistent compression. The remaining six rats that suffered from the laminectomy at the same level served as controls (n=6). The animal was then allowed to

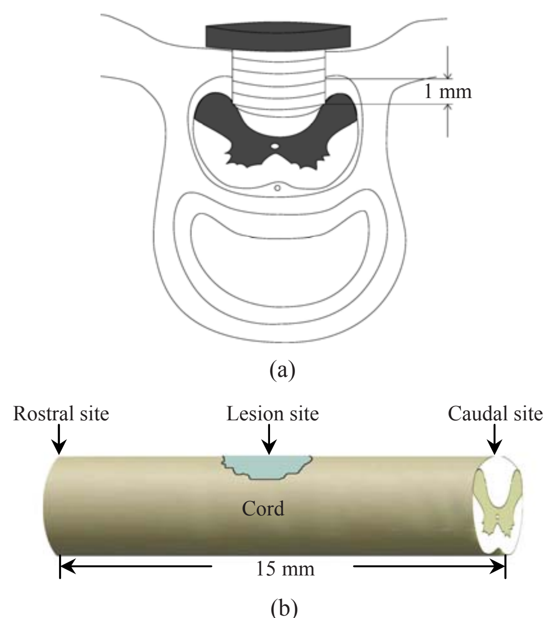

Fig.1 A schematic drawing of the mechanical compressive SCI animal model. (a) A custom-made mini-screw applied for the compression of the spinal cord through the dura mater, vertically and dorsally until 1 mm of the screw tip is inserted; (b) The transverse sections prepared at the lesion center, 7.5 mm rostral and caudal from the center

recover from anaesthesia and housed under a 12 h-12 h light-dark cycle in a bio-clean room with free *per os* access to water and food.

All surgeries and animal care strictly followed the Guidelines and Policies for Rodent Survival Surgery of the University (School of Medicine, Zhejiang University, China).

Immunohistochemical studies

Rats that were subjected to decompression and persistent compression were anaesthetised and then sacrificed on Day 1 or 3, or in Week 1 or 4 (n=6 at each time interval) using intracardiac perfusion with normal saline followed by 4% (w/v) paraformaldehyde in phosphate buffered saline (PBS) at pH 7.4. For histological evaluation, a 15-mm spinal cord segment with the site of injury at its centre was cut and placed overnight in the same fixative in a refrigerator at 4 °C, and further embedded in paraffin. Transverse sections (4 μm thick) were serially prepared from the lesion centre, 7.5 mm rostral and caudal from the centre (Fig.1b).

TUNEL staining

Paraffin-embedded cord sections were deparaffinized in two changes of xylene for 5 min each. The sections were rinsed sequentially in 100%, 95% and 75% ethanol before being incubated with 20 g/ml proteinase K (Roche Applied Science, Penzberg, Germany) for 5 min to strip off nuclear proteins. TUNEL was performed using in situ cell death detection kit (Lot No. 1-684-817, Roche Applied Science, USA). After rinsing in PBS twice for 10 min, the area around the sample was dried, and the sections were incubated with the TUNEL reaction mixture for 60 min at 37 °C in a humidified atmosphere in the dark. The slides were rinsed three times in PBS to stop the reaction. The sections were colorized with horseradish peroxidase after incubation with a secondary anti-fluorescein-peroxidase-conjugate (Converter-peroxidase) in a humidified chamber for 30 min at 37 °C, and then the sections were counterstained with haematoxylin.

Caspase-3, Bcl-2, and Bax staining

The paraffin-embedded spinal cord sections were deparaffinized using a standard procedure described above before rinsing in PBS three times for 10 min. After aspirating the excess liquid from the slides, the sections were incubated for 10 min in 0.1% (w/v) hydrogen peroxidase in methanol to quench endogenous peroxidase activity. The slides were rinsed twice in PBS each for 5 min and then incubated in normal non-specific goat serum for 10 min at room temperature, followed by overnight incubation with primary antibodies (caspase-3, Bcl-2, and Bax; Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4 °C. The sections were then rinsed in PBS three times each for 5 min, incubated with biotin-conjugated secondary antibody for 30 min, again rinsed in PBS for three times each for 5 min, and incubated with avidin biotin enzyme reagent for 30 min. The sections were colorized with 3,3′-diaminobenzidine tetrahydrochloride in PBS at pH 7.4 containing 0.01% (v/v) H_2O_2, immersed in PBS to stop the reaction when the desired stain intensity appeared, counterstained with haematoxylin, and mounted according to the standard procedures.

Neural cell count

Three individuals including a pathologist not participating in the study counted the number of neural cells labelled by TUNEL staining in the posterior column (pc), lateral column (lc), anterior column (ac), anterior horns (ah), and posterior horns (ph) at three axial levels: Site a, the level 7.5 mm rostral to the centre of the lesion site; Site b, the centre of the lesion site; and Site c, the level 7.5 mm caudal to the centre of the lesion site. All sections were examined under an Olympus BX51 microscope (Olympus, Tokyo, Japan) and photographed by a DP11 digital camera (Olympus). The number of positive cells in white matters (WM) was calculated as the sum of pc, lc and ac, and in grey matters (GM) as the sum of ah and ph, respectively.

Statistical analysis

Data are expressed as mean±*SD*. The numbers of positive cells in the WM and GM were compared among the three experimental groups (decompression at 6 h, 24 h and persistent compression) using one-way analysis of variance (ANOVA) (Bonferroni's method) with the SPSS 11.0 version software on an IBM Thinkpad 2672GCC computer (Armonk, NY, USA). Differences were considered statistically significant at the level of $P<0.05$.

RESULTS

Histopathology of the sham controls

There were no histopathological abnormalities in sham-operated rats. The TUNEL-, caspase-3-, Bcl-2- and Bax-positive cells were sparse in both the GM and the WM. The topographic distribution of these cells was similar at the rostral site (Site a), lesion center (Site b) and caudal site (Site c).

Topographic distribution of TUNEL-positive cells

Fig.2 shows that the changes in the distribution of TUNEL-positive cells at Sites a, b and c from Day 1 to Week 4 after decompression in Groups A, B and C. In general, there were some similar changes among these three groups. The number of TUNEL-positive cells increased on Day 1 after decompression at Site b, but there were only a few such cells at Sites a and c at that time. From Day 3 to Week 4 after decompression, the number of TUNEL-positive cells decreased gradually at Site b, while they tended to increase at Sites a and c in the WM of all three groups. The peak numbers of TUNEL-positive cells in GM area at Site b were noted on Day 1 after decompression, while the peak numbers of TUNEL-positive cells in WM at Sites a and c were in Week 1 after decompression. In Week 4 after decompression, the TUNEL-positive cells were still detected at all the three sites in all the three groups but their numbers decreased. We also found that the number of the TUNEL-positive cells in the sham controls was so small, so we did not put them into the statistical analysis.

On Day 1 after decompression, a significant decrease of TUNEL-positive cells was observed at WM and GM areas of Site b (*P*=0), and a significant reduction of these cells was still noted at this site on Day 3 post-decompression, and especially by 6 h after decompression (*P*=0.03). On the other hand, a significant decrease of TUNEL-positive cells was observed at WM and GM areas at Site c (*P*=0.01). In Week 1 after decompression, a significant reduction of TUNEL-positive cells was still noted at Site b (*P*=0.02), while a significant decrease of these cells was observed in the WM of the peripheral sites, especially by 6 h after decompression (*P*=0 at Site a; *P*=0.01 at Site c). In Week 4 after decompression, a significant reduction of TUNEL-positive cells was observed at Site b (*P*=0.02), as well as at Sites a (*P*=0.01) and c (*P*=0.03) (Fig.3).

Caspase-3, Bcl-2 and Bax immunohistochemistry

We also stained the spinal cord tissues for caspase-3, Bcl-2 and Bax to confirm the TUNEL findings. Changes in immunoreactivity were examined in the GM between Day 1 and Week 4 after injury. In general, the numbers of caspase-3- and Bax-positive cells were similar to those of TUNEL-positive cells. In contrast, a reverse pattern was found with regard to the number of Bcl-2-positive cells. Fig.4 shows a typical example of staining for TUNEL, caspase-3,

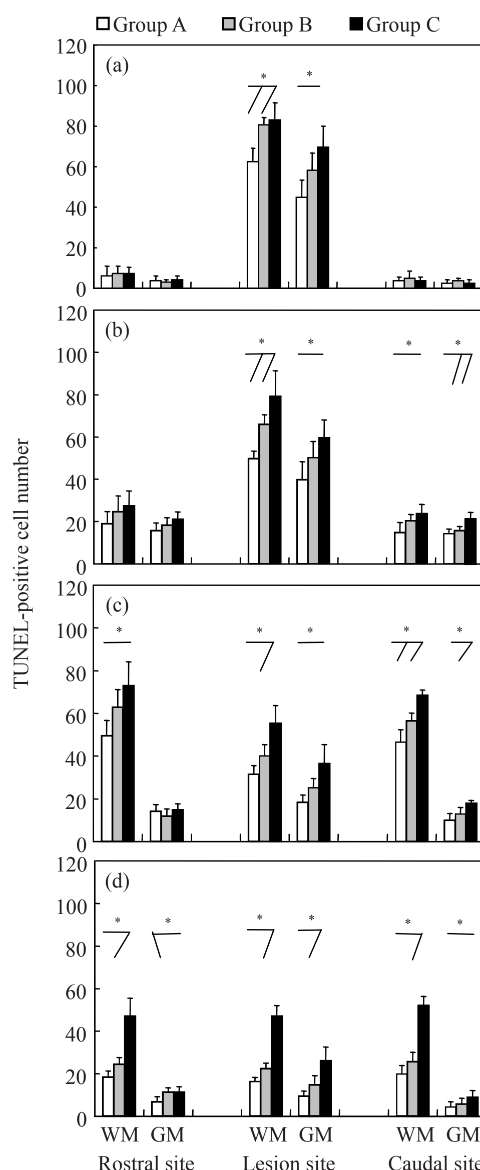

Fig.2 Topographic distribution of TUNEL-positive cells. This figure shows the topographic distribution of TUNEL-positive cells on Day 1 (a) and Day 3 (b), and in Week 1 (c) and Week 4 (d) after decompression at the rostral, lesion and caudal sites of the spinal cord. Data are mean±*SD*. $^*P$<0.05

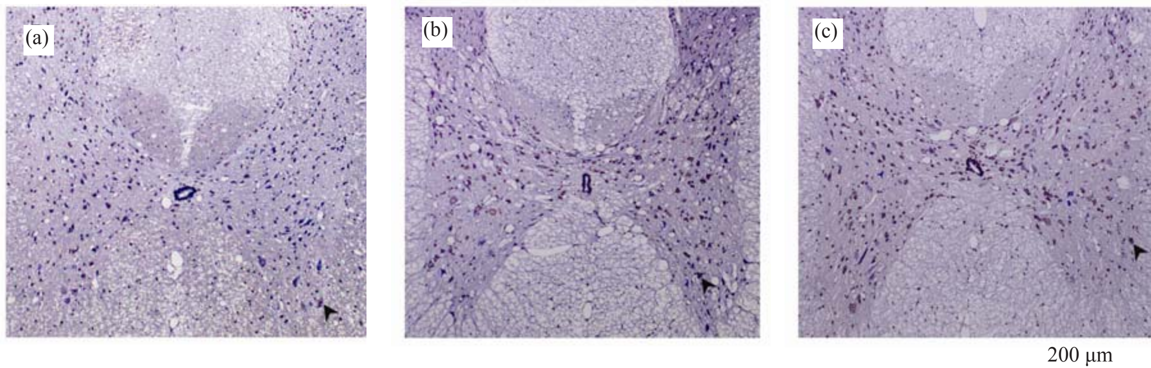

Fig.3 Photomicrographs of the TUNEL-positive cells of three different groups at the rostral site. This figure showing significant decrement in the number of TUNEL-positive cells at a spinal cord level rostral to the lesion site (TUNEL staining). (a) Group A (decompression at 6 h after compression); (b) Group B (decompression at 24 h after compression); (c) Group C (persistent compression). Black arrow heads show the TUNEL-positive cells staining black brown in the nuclear

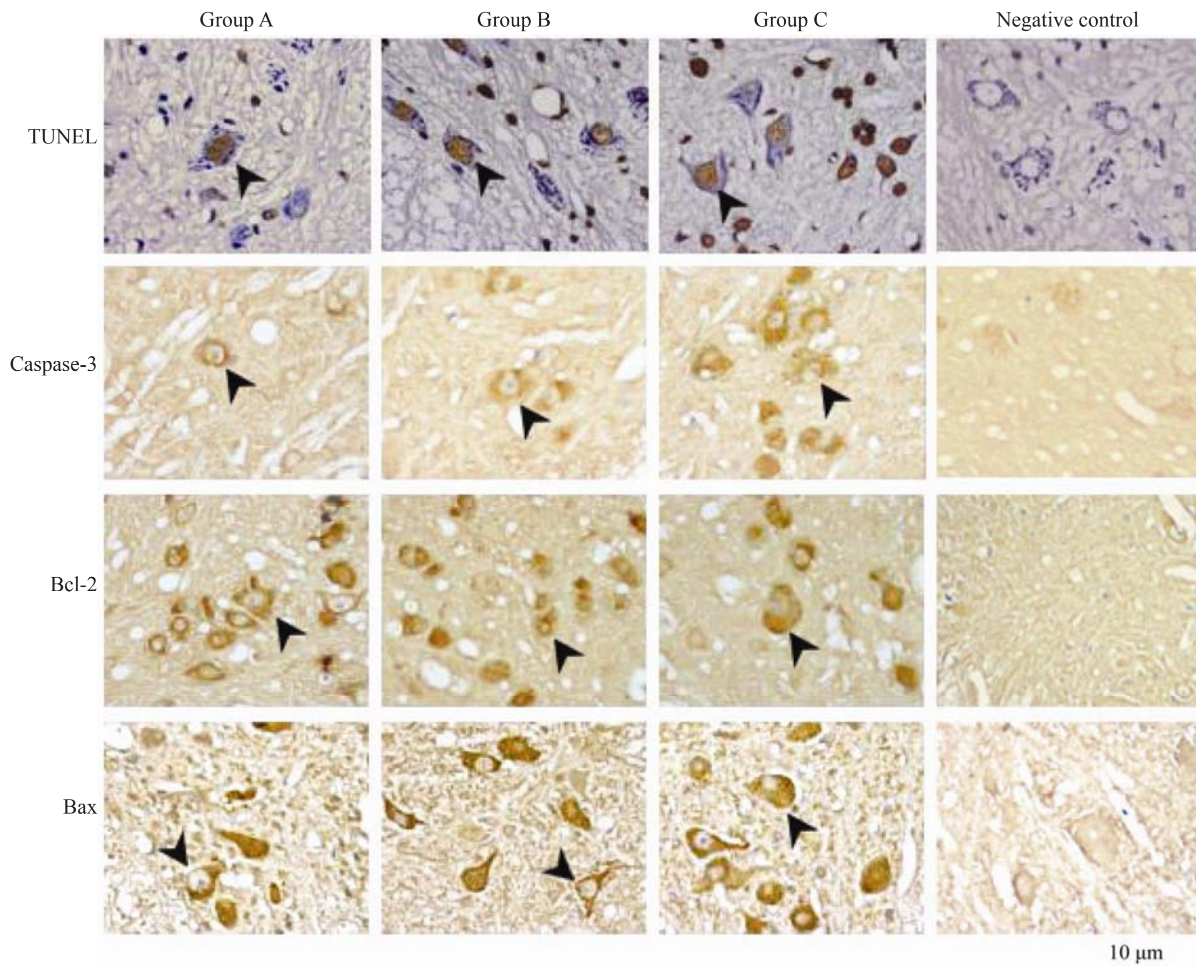

Fig.4 Representative sample of the immunohistochemical staining. Photomicrographs show TUNEL-, caspase-3-, Bcl-2- and Bax-stained cells in the anterior horn of the spinal cord at the level rostral to the site of the lesion in representative rats of Groups A, B, C, and negative control. Black arrows show the positive stained cells in Groups A, B, and C

Bcl-2, and Bax in Week 4 after the injury in the anterior horn of Site c of Groups A, B, and C. At that stage, the numbers of TUNEL-, caspase-3-, and Bax-positive cells were significantly lower in Groups A and B than in Group C. However, the numbers of Bcl-2-positive cells were significantly higher in Groups A and B.

DISCUSSION

Apoptosis in traumatic spinal cord injury

Recent studies have shown that apoptosis plays an important role in determining neurological outcome following SCI (Crowe *et al*., 1997; Emery *et al*., 1998). It is well known that neural cell loss in SCI occurs both at the time of injury and secondarily over a period of days to weeks after the traumatic event. Neuronal cell death occurs mainly by necrosis; however, it continues throughout a much more extensive axial section in the spinal cord WM for a period of up to several weeks in a process referred as secondary injury (Katoh *et al*., 1996; Crowe *et al*., 1997; Liu *et al*., 1997; Springer *et al*., 1999; Saito *et al*., 2000). In these studies, visible signs of oligodendrocyte apoptosis appear within 24 h and continue for at least 3 weeks after injury. A histological study of human SCI reported the existence of apoptotic oligodendrocytes from 3 h to at least 8 weeks after the injury (Emery *et al*., 1998).

In the present study, we investigated apoptosis with special references to cellular morphology, nuclear chromatin staining with the TUNEL method, and immunoreactivity of three apoptosis-related proteins (caspase-3, Bcl-2, and Bax). We found that on Days 1 and 3 after spinal cord injury, the number of TUNEL-positive cells was high at the lesion site, whereas only a few of these cells were found at the sites rostral and caudal to the lesion. However, in Week 1 after injury, the number of TUNEL-positive cells decreased at the lesion site, but increased at the peripheral sites. In Week 4 post-SCI, the number of TUNEL-positive cells diminished at the rostral and caudal sites especially in the WM. These findings are in agreement with those of previous studies (Li *et al*., 1996; Liu *et al*., 1997), reflecting the natural history of the pathophysiology of SCI, and suggest that changes in the number of TUNEL-positive cells during SCI are related to apoptosis. The process of apoptosis is complex and involves the upregulation of numerous key molecules (Springer *et al*., 1999). Therapeutic benefits may be achieved by inhibiting such upregulation to prevent apoptosis.

Apoptosis secondary to spinal cord injury and effect of decompression

It is still not clear whether decompression surgery reduces neural cell apoptosis secondary to SCI. In the present study, we found that the number of TUNEL-positive cells in the lesion site was significantly reduced by decompression on Day 1 and Day 3, and in Week 1 and Week 4 after injury, compared with that of persistently-compressive group. Moreover, on Day 3 and in Week 1 and Week 4 after injury, the numbers of TUNEL-positive cells at the rostral and caudal sites were significantly lower in the spinal cord decompressed rats compared with that of the persistently-compressed rats. These findings suggest that decompression surgery suppresses the onset of neural cell apoptosis secondary to SCI. However, a serious drawback of TUNEL staining is that not all TUNEL-positive cells are apoptotic cells; DNA fragmentation also occurs in accidental cell death process. Thus, we studied caspase-3, Bcl-2 and Bax immunoreactivities in the GM. Our results confirm that the immunoreactivities of caspase-3 and Bax correlated with the changes noted in TUNEL-positive cells, indicating that the observed cellular changes reflect apoptosis of neural cells.

The genetic and environmental factors that trigger neuronal apoptosis may be different in various physiological and pathological settings. The biochemical alterations that occur during the early stages of apoptosis may induce mitochondrial dysfunction either directly or indirectly. The Bcl-2 family of proteins includes both pro- and anti-apoptotic members (Pellegrini and Strasser, 1999). The best-studied anti-apoptotic member in neurons is Bcl-2, and pro-apoptotic members include Bax- and Bcl-associated death promoter. Previous studies showed that overexpression of Bcl-2 in cell cultures and in transgenic mice increases resistance of neurons to death induced by excitotoxic, metabolic and oxidative insults relevant to stroke and other disorders (Martinou

et al., 1994). Conversely, neurons lacking Bax are protected against apoptosis (White *et al*., 1998). Other mechanisms that regulate the early stages of apoptosis include various caspases, the prostate apoptosis response-4 protein and telomerase. Some caspases are activated during the early phase of apoptosis, such as caspase-3. Activation of caspase-3 enhances the cleavage of DNA, nuclear lamins, cytoskeletal components and proteins that inhibit apoptosis (Hengartner, 2000).

While several mechanisms could explain the effect of decompression on the recovery of SCI, we demonstrated in the present study that decompression results in reduction of TUNEL-positive cells following SCI, especially in the early period following decompression (6 h). This decompression-related mechanism might positively improve the clinical outcome after SCI.

CONCLUSION

The beneficial effects of decompression of the mechanically-injured spinal cord may reflect, at least in part, changes in the apoptotic cell population at the site of the lesion. Surgical decompression may have favourable effects on the outcome of SCI by reducing the number of apoptotic cells at the lesion site as well as the surrounding areas.

References

Aki, T., Toya, S., 1984. Experimental study on changes of the spinal-evoked potential and circulatory dynamics following spinal cord compression and decompression. *Spine*, **9**(8):800-809. [doi:10.1097/00007632-198411000-00006]

Baydin, A., Cokluk, C., Aydin, K., 2007. A new minimally invasive experimental spinal cord injury model in rabbits. *Minim. Invasive Neurosurg.*, **50**(3):170-172. [doi:10.1055/s-2007-985375]

Carlson, G.D., Minato, Y., Okada, A., Gorden, C.D., Warden, K.E., Barbeau, J.M., Biro, C.L., Bahnuik, E., Bohlman, H.H., Lamanna, J.C., 1997. Early time-dependent decompression for spinal cord injury: vascular mechanisms of recovery. *J. Neurotrauma*, **14**(12):951-962. [doi:10.1089/neu.1997.14.951]

Crowe, M.J., Bresnahan, J.C., Shuman, S.L., Masters, J.N., Beattie, M.S., 1997. Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. *Nat. Med.*, **3**(1):73-76. [doi:10.1038/nm0197-73]

Delamarter, R.B., Sherman, J., Carr, J.B., 1995. Pathophysiology of spinal cord injury: recovery after immediate and delayed decompression. *J. Bone Joint Surg. Am.*, **77**(7):1042-1049.

Dimar, J.R., Glassman, S.D., Raque, G.H., Zhang, Y.P., Shields, C.B., 1999. The influence of spinal canal narrowing and timing of decompression on neurologic recovery after spinal cord contusion in a rat model. *Spine*, **24**(16):1623-1633. [doi:10.1097/00007632-199908150-00002]

Dolan, E.J., Tator, C.H., Endrenyi, L., 1980. The value of decompression for acute experimental spinal cord compression injury. *J. Neurosurg.*, **53**(6):749-755.

Emery, E., Aldana, P., Bunge, M.B., Puckett, W., Srinivasan, A., Keane, R.W., Bethea, J., Levi, A.D., 1998. Apoptosis after traumatic human spinal cord injury. *J. Neurosurg.*, **89**(6):911-920.

Guha, A., Tator, C.H., Endrenyi, L., Piper, I., 1987. Decompression of the spinal cord improves recovery after acute experimental spinal cord compression injury. *Paraplegia*, **25**(4):324-339.

Hengartner, M.O., 2000. The biochemistry of apoptosis. *Nature*, **407**(6805):770-776. [doi:10.1038/35037710]

Kasahara, K., Nakagawa, T., Kubota, T., 2006. Neuronal loss and expression of neurotrophic factors in a model of rat chronic compressive spinal cord injury. *Spine*, **31**(18):2059-2066. [doi:10.1097/01.brs.0000231893.21964.f2]

Katoh, K., Ikata, T., Katoh, S., Hamada, Y., Nakauchi, K., Sano, T., Niwa, M., 1996. Induction and its spread of apoptosis in rat spinal cord after mechanical trauma. *Neurosci. Lett.*, **216**(1):9-12. [doi:10.1016/0304-3940(96)12999-2]

Li, G.L., Brodin, G., Farooque, M., Funa, K., Holtz, A., Wang, W.L., Olsson, Y., 1996. Apoptosis and expression of Bcl-2 after compression trauma to rat spinal cord. *J. Neuropathol. Exp. Neurol.*, **55**(4):280-289. [doi:10.1097/00005072-199603000-00003]

Liu, X.Z., Xu, X.M., Hu, R., Du, C., Zhang, S.X., McDonald, J.W., Dong, H.X., Wu, Y.J., Fan, G.S., Jacquin, M.F., *et al*., 1997. Neuronal and glial apoptosis after traumatic spinal cord injury. *J. Neurosci.*, **17**(14):5395-5406.

Martinou, J.C., Dubois-Dauphin, M., Staple, J.K., Rodriguez, I., Frankowski, H., Missotten, M., Albertini, P., Talabot, D., Catsicas, S., Pietra, C., 1994. Overexpression of Bcl-2 in transgenic mice protects neurons from naturally occurring cell death and experimental ischemia. *Neuron*, **13**(4):1017-1030. [doi:10.1016/0896-6273(94)90266-6]

Nyström, B., Berglund, J.E., 1988. Spinal cord restitution following compression injuries in rats. *Acta Neurol. Scand.*, **78**(6):467-472. [doi:10.1111/j.1600-0404.1988.tb03689.x]

Panjabi, M.M., 1987. Experimental spinal cord trauma: a biomechanical viewpoint. *Paraplegia*, **25**(3):217-220.

Pellegrini, M., Strasser, A., 1999. A portrait of the Bcl-2 protein family: life, death, and the whole picture. *J. Clin.*

Immunol., **19**(6):365-377. [doi:10.1023/A:1020598632068]

Saito, N., Yamamoto, T., Watanabe, T., Abe, Y., Kumagai, T., 2000. Implications of p53 protein expression in experimental spinal cord injury. *J. Neurotrauma*, **17**(2): 173-182. [doi:10.1089/neu.2000.17.173]

Shuman, S.L., Bresnahan, J.C., Beattie, M.S., 1997. Apoptosis of microglia and oligodendrocytes after spinal cord contussion in rats. *J. Neurosci. Res.*, **50**(5):798-808. [doi:10.1002/(SICI)1097-4547(19971201)50:5<798::AID-JNR16>3.0.CO;2-Y]

Springer, J.E., Azbill, R.D., Knapp, P.E., 1999. Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. *Nat. Med.*, **5**(8):943-946. [doi:10.1038/11387]

Turker, M., Tezeren, G., Tukenmez, M., Percin, S., 2005. Indirect spinal canal decompression of vertebral burst fracture in calf model. *Arch. Orthop. Trauma Surg.*, **125**(5):336-341. [doi:10.1007/s00402-005-0816-4]

Wada, S., Yone, K., Ishidou, Y., Nagamine, T., Nakahara, S., Niiyama, T., Sakou, T., 1999. Apoptosis following spinal cord injury in rats and preventative effect of *N*-methyl-D-aspartate receptor antagonist. *J. Neurosurg. Spine*, **91**(1 Suppl.):98-104.

White, F.A., Keller-Peck, C.R., Knudson, C.M., Korsmeyer, S.J., Snider, W.D., 1998. Widespread elimination of naturally occurring neuronal death in Bax-deficient mice. *J. Neurosci.*, **18**(4):1428-1439.