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Effects of α-Phenyl-N-tert-butyl Nitrone (PBN) on Compression Injury of Rat Spinal Cord

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α-Phenyl-N-tert-butyl Nitrone (PBN) is a free radical scavenger which recently has proved to be neuroprotective in experimental studies on focal cerebral ischemia and infarction. We therefore studied the effect of this drug in a model of moderate compression injury to rat spinal cord at the midthoracic level. The compound was given intraperitoneally 0.5 h before (100 mg/kg b.w) and at 1.5 h (50 mg/kg b.w) and 3.5 h (50 mg/kg b.w) after compression. Treated animals and controls (vehicle alone) were allowed to survive for 1 or 9 days following trauma. The functional outcome was tested by the inclined plane method and the motor performance score. By using MAP2 immunostaining the number of nerve cell bodies in the ventral horn and the ratio of MAP2 immunostained area to area of whole section of the cord were assessed to detect loss of neurons and loss of dendrites in the compressed segment. βAPP and PGP9.5 immunostaining was used to demonstrate axonal lesions.

Treated and control rats showed at day 1 when tested with the inclined plane method a marked reduction of the capacity angle. This abnormality recovered gradually over the following days and was normalized at day 9. The motor performance score showed a marked reduction at day 1 which almost normalized at day 9. There was no difference regarding the functional outcome between rats given PBN and controls in none one of these functional tests.

The spinal cord of normal rats presented immunoreactivity to MAP2 in nerve cell bodies and dendrites but not in axons and other structures. Following compression there was at day 1 and 9 a marked loss of MAP2 immunoreactivity in dendrites and nerve cell bodies. We could not detect any difference between the PBN and the control rats regarding the degree of cell loss or degree of reduction of dendrite staining. No difference between the two groups was seen with the axonal immunostainings (βAPP and PGP9.5).

In conclusion, our study did not reveal any neuroprotective effect of PBN on the functional outcome and morphology (immunostaining to MAP2, βAPP and PGP9.5) in this model of moderate compression trauma to rat spinal cord.

Keywords: Behavoir tests, MAP2, PBN, rat, spinal cord, trauma

INTRODUCTION

Traumatic injuries to the cord can cause significant disability.[1,2] The functional deficits in severe cases such as paralysis, loss of sensory

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qualities and autonomic dysfunctions, are caused by the immediate mechanical injury (primary injury) and by secondary pathophysiological mechanisms of biochemical and circulatory origin.[3] The outcome following traumatic spinal cord injury has been clinically improved by drugs, particularly methylprednisolone, probably by interfering with factors mediating or maintaining secondary injuries.[4,5] Similar benificial effects has been shown in experimental trauma using lazaroids.[6]

In order to rescue as much as possible of functions after spinal cord trauma it is of interest to test new drugs which may reduce secondary injuries. Free radicals are considered to be key factors in the pathophysiology of secondary lesions of acute traumatic spinal cord injury and brain trauma.[7–11] It has been reported that in compression injury of rat spinal cord the levels of nitric oxide and lipid peroxides are elevated at the injured site and the region around the trauma.[12] Free radicals can be produced in a large amount upon phospholipid hydrolysis and by excitatory amino acid release.[7–11] The free radicals can induce lipid peroxidation which accelerates membrane damage leading to severe cell injury.[9,10] α-phenyl-N-tert-butyl nitrone (PBN) and lazaroids are both free radical scavengers.[6,13–15]

Recently, PBN has been extensively studied in experimental cerebral ischemia since several reports indicate that it has a considerably neuroprotective effect.[11,13–16] In studies of gerbil brain ischemia, PBN can attenuate forebrain edema and increase survival of CA1 neurons.[15] In addition, PBN can markedly reduce infarct size[14,16] and improve recovery of brain energy state in ischemia of rats.[13] Other experiments on ischemia in gerbils have revealed that PBN can lower brain oxidized protein levels.[17,18]

There is very limited information about the effects of PBN on CNS trauma. Sen *et al.*[11] reported that PBN inhibits free radical release in rat brain concussion. In addition, it has been found that pretreatment with PBN improves energy metabolism after spinal cord compression trauma to rats.[19]

We are interested in the pathophysiology and treatment of secondary lesions in spinal cord trauma. We use a rat model in which we can vary the degree of compression injury to the spinal cord from mild to severe.[20,21] In the present study, we evaluated the effects of a combination of pre- and post-treatment with PBN on moderate compression injury. To that end we applied microtubule-associated protein 2 (MAP2),[22] β-amyloid precursor protein (βAPP),[23] and protein gene product 9.5 (PGP 9.5)[24] immunohistochemistry which are useful methods for studies on cell bodies, dendrites and axons of the cord. Finally, we tested the functional outcome by the inclined plane method of Rivlin and Tator[25] and a combination of four tests, called motor performance score, proposed by Euler *et al.*[26] which is significantly correlated with loss of spinal cord tissue.

MATERIALS AND METHODS

Animals

Thirty-seven male Sprague-Dawley rats with an average weight of 310–340g were used. Food and water were provided ad libitum before and after the experiments. The rats were kept at a temperature of 20°C controlled thermostatically and exposed to alternate light and dark periods of 12 h.

Spinal Cord Injury

We used a rat model with compression injury of the spinal cord.[20,21] The animals were anaesthetised with a mixture of fluanisone 2.5 mg/ml and midazolam 1.25 mg/ml in distilled water in a total volume of 1.5 to 2 ml/kg of body weight, given subcutaneously. A catheter (PE 50) was inserted into the tail artery for continuous recording of the mean arterial blood pressure and sampling of blood. The rats were then placed in a

prone position on a heating pad and the body temperature was kept constant at about 37.5°C by using a rectal thermistor.

The laminae of Th_7 and Th_8 vertebrae, which overlie the Th_8 and Th_9 segments of the spinal cord, were removed leaving the dura intact. The animals were placed in a stereotactic frame with two adjustable forceps applied to the spinous processes of vertebrae cranial and caudal to the laminectomy in order to stabilise the spinal cord. A 35g weight was applied on the exposed dura for 5 min.

The inclusion criteria of the physiological parameters preceding the spinal cord injury were as follows: PO_2 >9.5 kPa, PCO_2 4.5–6 kPa, pH 7.35–7.45, mean arterial blood pressure >100 mm Hg. There was a transient short lasting increase of blood pressure during compression.

The animals were randomly divided into 5 experimental groups (Table I). Five rats were used as normal controls. Other rats were subjected to a compression injury with a load of 35g for 5 min on the exposed dura mater resulting in a moderate injury characterised by a transient paraparesis.[25,27] These rats were given intraperitoneal injection of PBN or saline and were allowed to survive for 1 or 9 days.

PBN Administration

Three dosages of PBN (20 mg/ml in 0.9% sterile saline; product no. B 7263, Sigma) were injected intraperitoneally to rats with spinal cord injury. The first dosage, 100 mg/kg b.w was given 30 min prior to compression because the concentration of PBN in CNS reaches a peak at this interval after intraperitoneal administration to normal rats.[28] Two additional dosages of 50 mg/kg b.w were given 1.5h and 3.5h after injury. For control, the same volume of saline was applied to other injured rats. PBN and saline solutions were coded and the study was performed blind.

TABLE I Survey over the different experimental groups and the number of animals in each group

Groups	Survival 1 day	Survival 9 days
Normal control	5	
PBN	8	8
Saline	8	8

Behavioral Tests

Inclined Plane Method: Every animal was checked by the inclined plane technique[25] prior to operation and every second day thereafter. The rats were placed transversely on an inclined plane and the angle at which they could maintain themselves for 5 sec was recorded and described as the "capacity angle".[20] We have used this method in the past in a series of investigations on graded compression trauma to rat spinal cord.[29–31]

Motor Performance Score: Recently, Euler *et al.*[26] presented a new way of describing the functional outcome in a rat model of photochemically induced lesion of the spinal cord. Each animal can be tested repeatedly and is given a "motor performance score" (MPS) based on the outcome of several individual tests, described below (Fig. 1).

Briefly, the rats are first tested in an open field and given a motor score (see below) according to their spontaneous activity. The rats obtaining a motor score of 3–5, are then checked by a beam walk test (see below). Based on this test, MPS of 4–10 can be obtained. If it is difficult to distinguish 4 or 5 of the motor score the righting reflex test is added. A MPS of 0 is given to rats getting a motor score of 0 and showing hyperactive withdrawal reflex. Below is a summary of how the four tests are performed. From Euler *et al.*[26]

♦ Motor score: The motor score was assessed by observing spontaneous activity of the rat on a 1 × 0.65 m paper-covered table for 1 min.

Score	Criteria
0	No movement of hindlimbs, no weight bearing
1	Barely perceptible movement of hindlimb, no weight bearing

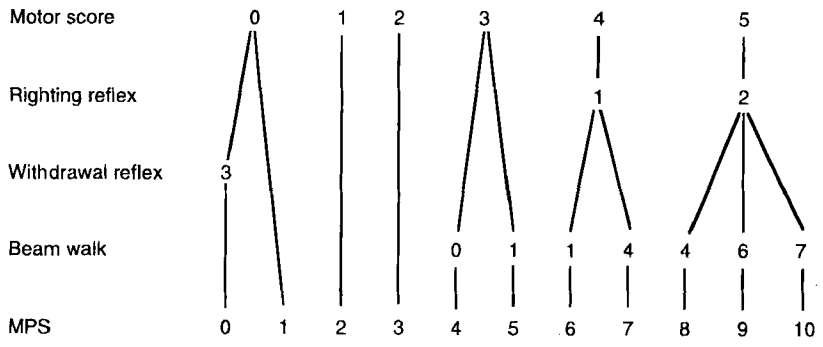

FIGURE 1 Outline of how the MPS scores are obtained based on four different tests. From Euler *et al.*[26]

2	Movement in hindlimb, no weight bearing
3	Can support weight on hindlimb
4	Walks with only mild deficit
5	Normal Walking

♦ Righting reflex: Rat was held in one hand and turned over on its back. It was released from a height of 7–8 cm above a covered table. The way the rat tried to regain its original position with its feet down was observed.

Score	Criteria
0	No attempt to right itself
1	Weak or delayed attempt to right or rights in the direction of the roll
3	Normal righting counter to the direction of roll

♦ Withdrawal reflexes: The hindlimb was withdrawn when stimulated by pulling the hindlimb backwards, pricking the sole of the foot with a needle and pressing the foot between the testers. The speed and force by which the hindlimb was withdrawn were tested.

Score	Criteria
0	No withdrawal
1	Weak withdrawal
2	Normal withdrawal
3	Hyperactive withdrawal

♦ Beam walk: The beam walk was tested by using four planks of different widths (1.7, 4.7, 6.7, 7.7 cm) but same length (1.5 m). Rats were placed on the widest plank and the capacity to walk on the plank without foot slips within two trials were observed. The procedure was repeated on successively narrower planks. The narrowest plank a rat could walk was recorded.

Score	Criteria
1	7.7 cm
4	4.7 cm
6	2.7 cm
7	1.7 cm

Sampling and Immunohistochemical Technique

When sacrificed, the animals were sedated with the same anaesthetics used during the spinal cord injury procedure and perfused through the heart with 200 ml of a phosphate buffer solution at a flow rate of 40 ml per min (PBS, pH 7.4) followed by 200 ml of a 4% formaldehyde solution in the same buffer at 100 mm Hg. The spinal cords were removed and samples from the Th_{8-9} segments were kept in fixative over night, dehydrated and samples were embedded in paraffin blocks. Three transverse sections were randomly taken from twenty sections which were cut from each segment and were stained by each of the following methods: hematoxylin-eosin, immunostaining with MAP2, βAPP and PGP9.5 antibodies.

After deparaffination, the sections were treated in the following order: microwave oven for 10 min in citrate buffer pH 6.0, in 1% H_2O_2 in methanol for 30 min, 1% BSA in PBS buffer for 30 min and then

incubated over night with different antibodies: the monoclonal antibody against MAP2 at a dilution of 1:1000 (Amersham International plc, Amersham, UK, code RPN 1194, batch 10), the monoclonal antibody against βAPP at a dilution of 1:100 (Boehringer Mannheim Biochemical clone 22C11) and a rabbit antiserum against human PGP 9.5 at a dilution of 1:2 500 (code no. RA 95101, Chemicon, Temecula CA).

The sections incubated with antibodies against MAP2 or βAPP were then exposed to rabbit anti-mouse IgG. Swine anti-rabbit IgG was used to the sections incubated with antiserum against PGP 9.5 for 30 min. The reaction product was visualised by the avidin-biotin-peroxidase complex method using 3,3,-diamino-benzidine tetrahydrochloride as the chromogen (Vectastain Elite Kit, Vector Laboratories, Burlingame, CA). To intensify the reaction product we applied the nickel enhancement procedure combined with the glucose-glucose oxidase method.[32–34] For control, the primary antibody was omitted and thereafter the sections were treated as those in which the primary antibodies had been applied.

Morphometric Measurement and Count of Neurons

Three sections, randomly from each segment were immunostained for MAP2 and used for morphometry. A computer program, IMP (Center of Image Analysis, Uppsala University) was used to measure the MAP2 immunostained area and the area of whole section of the cord. The ratio between them was used to assess the degree of MAP2 lesion. The number of stained nerve cell bodies was counted in each section stained with MAP2 immunohistochemistry.

Statistics Processing

Data obtained from inclined plane test, MPS, morphometric measurement of MAP2 immunohistochemistry and number of nerve cell bodies were statistically processed by using the computer program Statview 4.01, Abacus Concepts. For comparison of multiple means between groups factorial analysis of variance was used. Fisher's protected least squares difference (PLSD) test was performed for post hoc testing. Values in Figures are given as the mean ± SEM. Differences with a p-value <0.05 were considered significant.

RESULTS AND COMMENTS

As shown below the compression caused functional changes in the same way as has been reported earlier in this model of spinal cord compression.[21,27,29,35] The impact also induced a number of pathological changes of the cord at the site of compression of the same character as described in our earlier papers.[21,27,29,36] Thus, at day 1 we could observe scattered small bleedings at the surface of the cord; this change disappeared at day 9. Haematoxylineosin stained sections of the compressed segment displayed at day 1 multiple small bleedings in the grey matter of the cord. Some nerve cell bodies were condensed. The longitudinal tracts contained expanded axons and vacuoles.

♦ Behavioral Tests

Inclined Plane: Compression injury caused a pronounced impairment at day 1 but within 5 or 7 days the value of the capacity angle had returned close to the normal level. The value of the angle even exceeded the normal level at day 9 after compression (Fig. 2, upper). There was no statistical difference between groups given PBN treatment and non-treatment controls at any time point ($P > 0.05$).

MPS: Control rats obtained a value of 10 in the motor performance score. Only rats with an initial score of 10 were included in the groups of rats subjected to spinal cord injury. At day 1 after compression, MPS was less than 7 and there was a recovery over the following days in the same way as observed with the inclined plane method

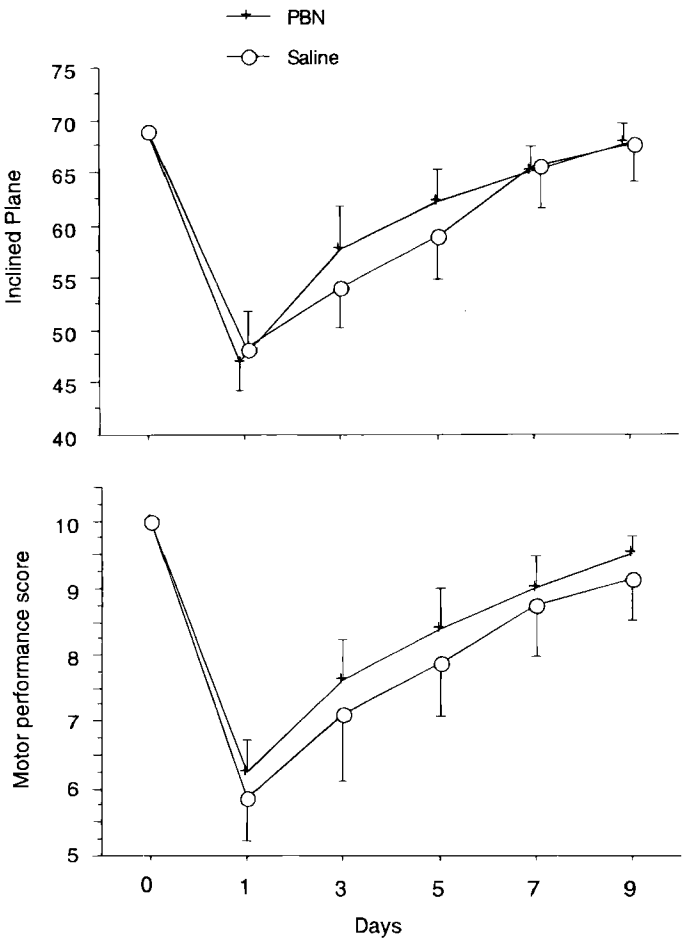

FIGURE 2 Graphs showing the changes of neurological functions tested by the inclined plane method (upper) and motor performance score (lower) in PBN treated rats and non-treatment controls after spinal cord injury. Day 0 = pre-injury, Bars:±SEM.

(Fig. 2, lower). No significance was seen regarding means of MPS score between treated and non-treated groups at any time point after compression ($P > 0.05$).

♦ Morphometric Analysis of MAP2 Immunoreactivity and Count of Neurons

The spinal cord of normal rats showed MAP2 immunoreactivity in dendrites and nerve cell bodies of the grey matter (Fig. 3). Usually, the dendrites were more heavily stained than the cell bodies. In both of groups (PBN treatment and non-treatment), the compressed Th_{8-9} segment showed by light microscopy loss of immunoreactivity in nerve cell bodies and dendrites, particularly in the ventral horns at day 1 and 9 after injury (Fig. 4).

MAP2 immunoreactive areas were measured by computer image analysis (Fig. 5, upper). The ratio of MAP2 immunostained area of the grey matter to the whole section area was used to assess the degree of severeity of the lesion. At day 1 after compression, there was compared with controls a marked reduction of MAP2 stained area in the grey matter of the cord ($P < 0.0001$). At day 9, there was no obvious recovery of MAP2 staining compared with the findings at day 1 ($P > 0.05$).

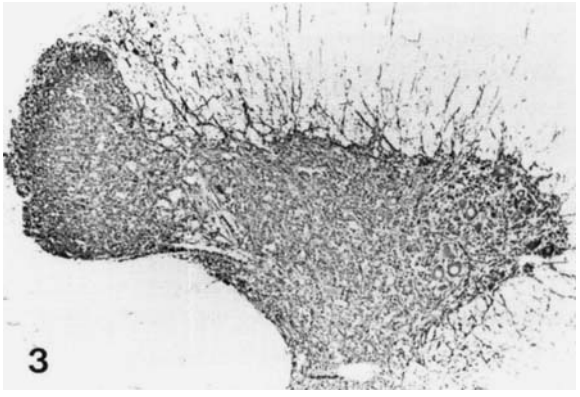

FIGURE 3 MAP2 immunoreactivity in normal spinal cord. Reactivity is seen in dendrites and nerve cell bodies of the grey matter. The dendrites are more heavily stained than the cell bodies. No immunoreactivity is present in axons and non-neuronal cells.

We also counted the number of labelled nerve cell bodies in the ventral horns in sections stained with MAP2 immunohistochemistry. Both groups showed a reduction of labelled nerve cell bodies. This reduction was of about the same order at day 1 as at day 9 after trauma. We could not find any statistical significant difference between PBN treated and control rats regarding the loss of nerve cell bodies (Fig. 5, lower).

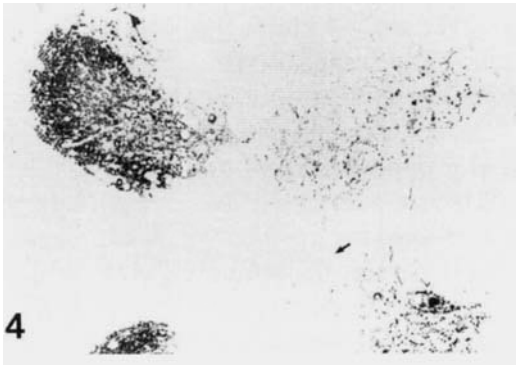

FIGURE 4 Changes of MAP2 immunoreactivity in compressed spinal cord at day 1 after injury. There is a loss of immunoreactivity in some areas of the grey matter. The arrow points the central canal.

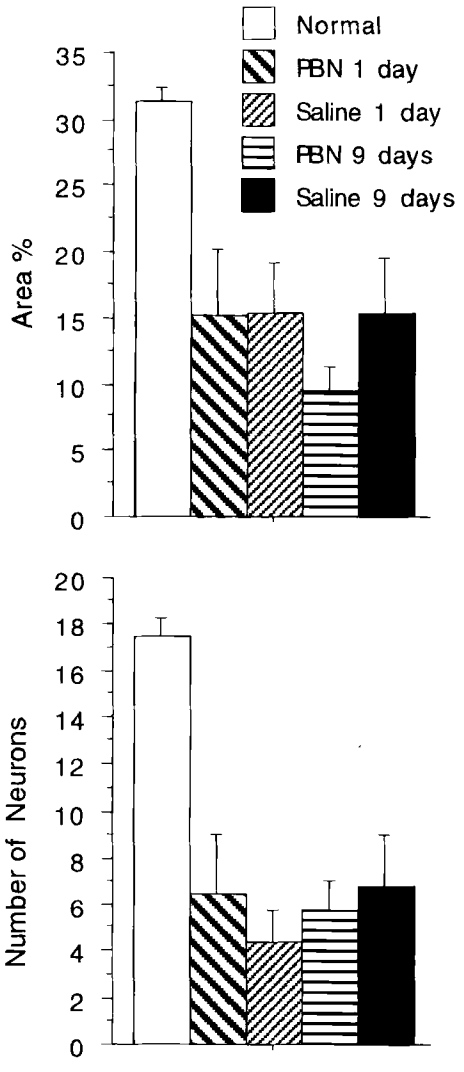

FIGURE 5 Graphs illustrating the loss of MAP2 immunoreactivity measured by computer image analysis (upper) and the reduction of number of nerve cell bodies (lower) (PBN treated rats and saline controls at day 1 and 9). Bars:±SEM.

♦ βAPP and PGP9.5 Immunoreactivity

The spinal cord from normal controls presented βAPP immunoreactivity in nerve cell bodies and the initial part of a few axons. The axons of the white matter were not stained. Scattered immunoreactive glial cells of the white matter were detected. The compressed segment of injured animals showed at day 1 after trauma many markedly swollen axons with intensely

βAPP immunoreactivity in the white matter. At day 9, similar marked changes were present. In addition, there were numerous small, closely located, rounded profiles in the white matter particularly in the corticospinal tract.

The nerve cell bodies of normal spinal cord presented weak PGP 9.5 immunostaining of the cytoplasm. Glial cells were not labelled. Axons of the longitudinal tracts were weakly stained but the corticospinal tracts were free from staining. The PGP9.5 immunoreactive axons were numerous in the longitudinal tracts of the injured segment but they were never found in the corticospinal tracts following compression. Axonal swellings showed more extensive PGP9.5 staining than axons of normal size. At day 9, many swollen axons with PGP9.5 immunoreactivity were still present. A few nerve cell bodies showed positive staining.

No obvious difference was found regarding the extent of βAPP and PGP9.5 changes between groups with PBN treatment and non-treated injured rats.

DISCUSSION

Several recent reports indicate that the free radical trapping agent, PBN[11,37–39] has remarkable neuroprotective effects in studies of brain ischemia.[11,13–18] The great current interest in this compound is also due to the fact that it has proved to have effects in some experimental situations even when it is given a few hours after ischemia.[13,14,16]

Free radicals are thought to be cardinal factors in the secondary processes not only of ischemic brain lesions but also of acute traumatic CNS injury.[7–11] Previously, there is only one published paper on PBN treatment in experiments on traumatic injury to the CNS. Thus, Sen *et al.*[11] reported that PBN inhibits free radical release in rat brain concussion. This fact encouraged us to start studies on the effects of PBN on compression injury of rat spinal cord. However, with the experimental set-up applied, the dosages used and the outcome measures employed we could not find any clear-cut positive effect of PBN treatment.

We used a compression model of rat spinal cord in which the degree of lesion and the survival period can be varied. [29–31] By using moderate compression we induced a lesion characterised by transient loss of motor functions tested by the inclined method and by the motor performance score techniques.[26] The compression induced local pathological changes of the grey matter of the cord as well as moderate lesions of axons of the longitudinal tracts. Such morphological changes were present from day 1 to day 9, i.e. throughout the entire experimental period. There is thus a discrepancy between functional restoration and morphological changes which persist at least to day 9 in this model of moderate compression. The degree of injury which we produced appears to be adequate for this study. The absence of any recorded positive effects of PBN does not exclude the possibility that the compound may have some consequences in other types of trauma to the cord.

Theoretically, the absence of any PBN effects may have something to do with the way the compound was given or with dose used. PBN was given intraperitoneally to the rats 30 min prior to compression, i.e. the commonly used route,[13,14,15,16] implying that concentration in the spinal cord would be at its peak. Two additional doses were given 1.5h and 3.5h after injury in order to maintain high PBN concentration in the tissue for about 6h; the biological half-life time is 134 min.[28] The dosage used, i.e. 100 mg/kg and 50 mg/kg are those used in previous investigations in which positive effects of PBN has been found in experimental cerebral ischemia.[11,13,14] The lack of any detectable effects in our study on spinal cord compression therefore does not seem to be due to the way the compound was given or to the dose level used.

We applied one traditional (inclined plane) and one new (motor performance score) method to assess the functional outcome after trauma and to record any effects of PBN. Both tests reflect the degree of impact to the cord and the functional changes over time after an injury can be

recorded.[26,27,36] In our study, the tests revealed expected functional deficits but there was no difference between rats given PBN and the controls. Even though these tests are among the best of available techniques to assess functional outcome they are fairly crude. Therefore, some positive effects of PBN in spinal cord compression trauma can not be ruled out.

The morphological methods used (MAP2, βAPP and PGP9.5 immunostainings) are recently developed techniques which are very useful to detect changes in nerve cell bodies, dendrites and axons of rat spinal cord after compression trauma.[22,23,24] MAP2 is a component in nerve cells, dendrites and MAP2 immunostaining is therefore very useful to detect injuries of these structures of the cord. Previously, most investigations using this technique, have been based on light microscopic observations alone but in a recent study of focal ischemia image analysis was applied to obtain quantitative information.[22] By using computer image analysis on coded sections we could not in the present study detect any PBN-induced reduction in the degree of injury to the nerve cells bodies and dendrites of the compressed segment. Nor did we observe any influence by PBN on the degree of axonal lesions. However, detection of degree of axonal lesions is very difficult since such changes are not suitable for morphometric analysis.

The absence of recorded effects of PBN in the present study was unexpected in light of the many recent studies demonstrating a neuroprotective effect of this compound in cerebral ischemia. [11,13–16] The lack of recorded positive effects may be due to methodological or biological factors. Even though the functional and morphological outcome methods applied are among the best ones for this type of experiments, they obviously have their limitations. Before we conclude that PBN has no effects at all in spinal cord trauma, additional experiments must be performed, for instance variation of model including more severe trauma and change of PBN derivatives to more active compounds. In addition, other outcome measures may be considered. Considering biological factors, the possibility exists that the neuroprotective capacity by PBN varies between different regions of the nervous system, being more pronounced in the brain than in the spinal cord. This view, i.e that PBN is not universally protective, is supported by findings in experiments on heart ischemia demonstrating lack of protection by this compound.[40,41]

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References

[1] Hardman, J. M. (1991). Cerebrospinal trauma. In *Textbook of Neuropathology* (ed. R. L. Davis, D. M. Robertson), Williams & Wilkins, Baltimore, pp 962–989.
[2] Austin, G. M. (1983). Trauma. In *The Spinal Cord* (ed. G. M. Austin), Igaku-Shoin Medical Publishers, New York, pp 215–261.
[3] Tator, C. H. (1995). Update on the pathophysiology and pathology of acute spinal cord injury. *Brain Pathology*, **5**, 407–413.
[4] Hall, E. D. (1992). The neuroprotective pharmacology of methylprednisolone. *Journal of Neurosurgery*, **76**, 13–22.
[5] Hall, E. D. (1993). Neuroprotective actions of glucocorticoid and nonglucocorticoid steroids in acute neuronal injury. *Cellular and Molecular Neurobiology*, **13**, 415–432.
[6] Clark, W. M., Hazel, J. S. and Coull, B. M. (1995). Lazaroid, CNS pharmacology and current research. *Drugs*, **50**, 971–983.
[7] Demediuk, P., Saunders, R. D., Clendenon, N. R., Means, E. D., Aderson, D. K. and Horrocks, L. A. (1985). Changes in lipid metabolism in traumatized spinal cord. *Progress in Brain Research*, **63**, 211–226.
[8] Faden, A. I., Chan, P. H., and Longar, S. (1987). Alterations in lipid metabolism, Na^+,K^+-ATPase activity, and tissue water content of spinal cord following experimental traumatic injury. *Journal of Neurochmistry*, **48**, 1809–1816.
[9] Hall, E. D., and Braughler, J. M. (1986). Role of lipid peroxidation in post-traumatic spinal cord degeneration: a review. *Central Nervous System Trauma*, **3**, 281–294.
[10] Hall, E. D. and Braughlar, J. M. (1993). Free radicals in CNS injury. *Res Publ Assoc Res Nerv Ment Dis*, **71**, 81–105.
[11] Sen, S., Goldman, H., Morehead, M., Murphy, S. and Phillis, J. W. (1984). Alpha-Phenyl-tert-butyl-nitrone inhibits free radical release in brain concussion. *Free Radicals in Biology and Medicine*, **16**, 685–691.

[12] Hamada, Y., Ikata, T., Katoh, S., Tsuchiya, K., Niwa, M., Tsutsumishita, Y. and Fukuzawa, K. (1996). Roles of nitric oxide in compression injury of rat spinal cord. *Free Radical Biology and Medicine*, **20**, 1–9.

[13] Folbergrova, J., Zhao, Q., Katsura, K. I., and Siesjo, B. K. (1995). N-tert-butyl-alpha-phenyl-nitrone improves recovery of brain energy state in rats following transient focal ischemia. *Proceedings of the National Academy of Sciences, USA*, **92**, 5057–5061.

[14] Cao, X. and Phillis, J. W. (1994). Alpha-Phenyl-tert-butyl-nitrone reduces cortical infarct and edema in rats subjected to focal ischemia. *Brain Research*, **644**, 267–272.

[15] Yue, T. L., Gu, J. L., Lysko, P. G., Cheng, H. Y., Barone, F. C. and Feuerstein, G. (1992). Neuroprotective effects of phenyl-tert-butylnitrone in gerbil global brain ischemia and in cultured rat cerebellar neurons. *Brain Research*, **574**, 193–197.

[16] Zhao, Q., Pahlmark, K., Smith, M. L. and Siesjo, B. K. (1994). Delayed treatment with the spin trap alpha-phenyl-N-tert-butyl-nitrone (PBN) reduces infarct size following transient middle cerebral artery occlusion in rats. *Acta Physiologica Scandinavica*, **152**, 349–350.

[17] Carney, J. M. and Floyd, R. A. (1991). Protection against oxidative damage to CNS by alpha-phenyl-tert-butly nitrone (PBN) and other spin-trapping agents: a noval series of nonlipid free radical scavengers. *Journal of Molecular Neuroscience*, **3**, 47–57.

[18] Floyd, R. A. and Carney, J. M. (1992). Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. *Annual Neurology*, **32 (Suppl)**, 22–27.

[19] Farooque, M., Olsson, Y. and Hillered, L. (1977). Pretreatment with α-phenyl-N-tert-butyl-nitrone (PBN) improves energy metabolism after spinal cord injury in rat. *Journal of Neurotrauma*, in press

[20] Nyström, B., Berglund, J. E., and Bergquist, E. (1988). Methodological analysis of an experimental spinal cord compression model in the rat. *Acta Neurologica Scandinavica*, **78**, 460–466.

[21] Holtz, A. (1989). Spinal cord compression injury. An experimental study in the rat with special reference to effects on spinal cord circulation. *Acta Universitatis Upsaliensis*, **209**, 1–43.

[22] Dawson, D. and Hallenbeck, J. (1996). Acute focal ischemia-induced alterations in MAP2 immunostaining: description of temporal changes and utilization as a marker for volumetric assessment of acute brain injury. *Journal of Cerebral Blood Flow and Metabolism*, **16**, 170–174.

[23] Sherriff, F. E., Bridges, L. R. and Sivaloganathan, S. (1994). Early detection of axonal injury after human head trauma using immunocytochemistry for β-amyloid precursor protein. *Acta Neuropathologica*, **87**, 55–62.

[24] Li, G. L., Farooque, M., Holtz, A. and Olsson, Y. (1997). Expression of the ubiquitin carboxyl-terminal hydrolase PGP 9.5 in axons following spinal cord compression trauma. An immunohistochemical study in the rat. *APMIS*, **105**, 384–390.

[25] Rivlin, A. and Tator, C. (1977). Objective clinical assessment of motor function after experimental spinal cord injury in the **rat**. *Journal of Neurosurgery*, **47**, 577–581.

[26] Euler, M., Åkesson, E., Samuelsson, E., Seiger, Å. and Sundström, E. (1996). Motor performance score: a new algorithm for accurate behavioral testing of spinal cord injury in rats. *Experimental Neurology*, **137**, 242–254.

[27] Li, G. L., Farooque, M., Holtz, A. and Olsson, Y. (1995). Changes of β-amyloid precursor protein after compression trauma to the spinal cord: An experimental study in the rat using immunohistochemistry. *Journal of Neurotrauma*, **12**, 269–277.

[28] Chen, G., Griffin, M., Poyer, J. and McCay, P. (1990). HPLC procedure for the pharmacokinetic study of the spin-trapping agent, alpha-phenyl-N-tert-butyl nitrone (PBN). *Free Radicals in Biology and Medicine*, **9**, 93–98.

[29] Farooque, M., Zhang, Y., Holtz, A. and Olsson, Y. (1992). Exudation of fibronectin and albumin after spinal cord injury in rats. *Acta Neuropathologica*, **84**, 613–620.

[30] Li, G. L., Brodin, G., Farooque, M., Funa, K., Holtz, A., Wang, W. L. and Olsson, Y. (1996). Apoptosis and expression of Bcl-2 after compression trauma to rat spinal cord. *Journal of Neuropathology and Experimental Neurology*, **55**, 280–289.

[31] Holtz, A., Nyström, B. and Gerdin, B. (1989). Spinal cord blood flow measured by ^{14}C-iodoantipyrine autoradiography during and after graded spinal cord compression in rats. *Surgical Neurology*, **31**, 350–360.

[32] Hancock, M. B. (1984). Visualization of peptide-immunoreactive processes of serotonin-immunoreactive cells using two color immunoperoxidase staining. *Journal of Histochemistry and Cytochemistry*, **32**, 311–314.

[33] Itoh, K., Konishi, A., Nomura, S., Mizuno, N., Nakamura, Y. and Sugimoto, T. (1979). Application of coupled oxidation reaction to electron microscopic demonstration of horseradish peroxidase: cobalt-glucose oxidase method. *Brain Research*, **175**, 341–346.

[34] Shu, S., Ju, G. and Fan, L. (1988). The glucose oxidase-DAB-nickel method in peroxidase histochemistry of the nervous system. *Neuroscience Letter*, **85**, 169–171.

[35] Holtz, A., Nyström, B. and Gerdin, B. (1990). Effect of methylprednisolone on motor function and spinal cord blood flow after spinal cord compression in rats. *Acta Neurologica Scandinavica*, **82**, 68–73.

[36] Li, G. L., Farooque, M., Holtz, A. and Olsson, Y. (1995). Microtubule-associated protein 2 as a sensitive marker for dendritic lesion after spinal cord trauma: An immunohistochemical study in the rat. *Restorative Neurology and Neuroscience*, **8**, 189–197.

[37] Imaizumi, S., Tominaga, T., Uenohara, H., Yoshimoto, T., Suzuki, J. and Fujita, Y. (1986). Initiation and propagation of lipid peroxidation in cerebral infarction models. Experimental studies. *Neurological Research*, **8**, 214–220.

[38] Sakamoto, A., Ohnishi, S. T., Ohnishi, T. and Ogawa, R. (1991). Protective effect of a new anti-oxidant on the rat brain exposed to ischemia-reperfusion injury: Inhibition of free radical formation and lipid peroxidation. *Free Radicals in Biology and Medicine*, **11**, 385–392.

[39] Tominaga, T., Imaizumi, S., Yoshimoto, T., Suzuki, J. and Fujita, Y. (1986). Detection of free radicals generated in NADPH-dependent lipid peroxidation in ischemia brain homogenates: use of the spin trapping technic. *No To Shinkei*, **38**, 169–175.

[40] Baker, J. E., Konorev, E. A., Tse, S. Y., Joseph, J. and Kalyanaraman, B. (1994). Lack of protection of PBN in isolated heart during ischemia and reperfusion: implications for radical scavenging mechanism. *Free Radical Research*, **20**, 145–163.

[41] Klein, H. H., Stier, A., Pich, S., Gehrke, D., Nebendahl, K., Lindert-Heimberg, S., Schade-Brittinger, C., Frode, R. and Schaper, J. (1993). Postischemic cell death in reperfused porcine hearts is not attenuated by the spin trap agent PBN during early reperfusion. *Basic Research in Cardiology*, **88**, 212–222.