Original Article

Post-traumatic moderate systemic hyperthermia worsens behavioural outcome after spinal cord injury in the rat

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Study design: A standardized rat model of compression spinal cord injury (SCI) was used to test the effect of transient systemic hyperthermia on long-term behavioural and morphometric outcomes.

Objective: To determine the effect of hyperthermia on the development of spinal cord lesion after SCI.

Setting: Institute of Neurobiology, Slovak Republic.

Methods: Male Wistar rats ($n = 30$) weighing between 300 and 330 g were used in the study. After incomplete spinal injury performed by balloon compression at the Th8–Th9 spinal level, rats were randomly divided into two groups. Rats in the treatment group were maintained hyperthermic for 3 h (rectal temperature at $40.5 \pm 0.5^\circ\text{C}$), immediately after SCI; rats from the control group received exactly the same procedure except that their rectal temperature was maintained at $37 \pm 0.5^\circ\text{C}$.

Results: The 3 h of post-traumatic hyperthermic treatment worsened behavioural outcome after SCI. Morphometric analysis showed that hyperthermia had a deleterious effect on white and grey matter, but the results did not reach statistical significance.

Conclusion: These results indicate that systemic hyperthermia exacerbates secondary processes in the lesion and significantly worsens behavioural outcome after traumatic SCI in the rat.

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Introduction

In CNS ischemia, body temperature has been recognized as the most effective intervention for modulating the outcome after ischemic injury. In general, hypothermia ameliorates and hyperthermia aggravates the extent of ischemic damage (for reviews see Corbett and Thornhill¹ and Ginsberg and Busto²). Based on this observation, careful monitoring of body temperature is one of the basic requirements in CNS ischemia experiments.³

Fever is a common complication in neurological patients, and hyperthermia represents a potential risk in all clinical situations in which either focal or global CNS ischemia may occur. The clinical importance of hyperthermia has been best documented after stroke, showing that increased body temperature is associated with worse neurological outcomes.^{4,5} The monitoring of body temperature and the prevention of hyperthermic episodes are part of routine care in stroke patients.^{2,6}

It can be expected that similarly to ischemia, body temperature variations will influence outcomes after traumatic CNS injury as well. The pathophysiology of both traumatic and ischemic injuries share several common mechanisms^{7,8} and ischemia due to vascular disturbances is one of the secondary processes in CNS trauma.⁹ In fact, significant effects of hypothermia on the outcome after traumatic brain injury (TBI) have been documented in experiments,¹⁰ and both systemic hypothermia and selective head cooling are being widely tested in clinical situations.^{11,12} However, both experimental and clinical studies have been mainly focused on the effects of mild hypothermic protection. The real effect of hyperthermia on the outcome after TBI has been studied systematically only in a few controlled animal experiments. In these studies, hyperthermia had either no effect ($40^\circ\text{C}/1\text{ h}$)⁷ or significantly worsened the outcomes ($39.5^\circ\text{C}/4\text{ h}$) as compared to normothermic animals.¹³ It is of interest that, in the latter study, morphological observations revealed the high

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sensitivity of myelinated axons to post-traumatic hyperthermia.

White matter vulnerability to ischemia/hyperthermia has also been observed in *in vitro* preparations, documenting that hyperthermia dramatically worsens anoxic injury to the isolated optic nerve.¹⁴ Taken together, these data indicate that myelinated axons are sensitive to ischemic damage, and their vulnerability increases with elevated temperature.

These observations support a hypothesis that a hyperthermic episode after traumatic spinal cord injury (SCI) might aggravate secondary damage to the spinal tissue and adversely affect neurological recovery in individuals with incomplete SCI. SCI is a specific case of CNS trauma, in which a permanent neurological deficit is caused primarily by damage to the white matter tracts. Clinical studies indicate that fever occurs in about 50% of patients after SCI.^{15–17} Fever is mostly caused by primary or secondary infections (mainly urinary tract infections) and thromboembolism.¹⁸ Patients with SCI at the higher thoracic or cervical levels exhibit partial poikilothermia because of the dissociation of thermoregulatory pathways.^{19,20} In rare cases, prolonged unexplained fevers occur in tetraplegic patients.^{16,21}

The present study sought to determine whether post-traumatic hyperthermia affects the outcome after SCI. This hypothesis has been tested by using a reproducible spinal cord compression model in the rat. The experimental conditions were designed to mimic relevant clinical situations with primary attention focused on the long-term neurological outcome.

Materials and methods

Spinal cord injury

In this study, 30 male Wistar rats were used. Their body weights ranged strictly between 300 and 330 g to ensure uniform anatomical measurements inside the spinal channel. Standard and reliable balloon compression model of SCI injury have been used in this study.²² After the induction of anaesthesia (4% halothane in room air), they were maintained under halothane anaesthesia (1–2% halothane in room air delivered via a face mask). Surgical procedures have been described in detail in our previous paper.²³ Briefly, the animal's back was shaved, and under aseptic conditions, a 2 cm midline skin incision was made to expose the L1–T10 spinous processes. With the aid of a dental drill and surgical microscope, a laminectomy was performed at the T10 level. The periosteal membrane was opened, and a 2F Fogarty catheter (Baxter Healthcare Corporation, Irvine, CA, USA) filled with saline and connected to a gas-tight Hamilton syringe was inserted into the epidural space cranially for 1 cm so that the centre of the balloon rested at the T8–T9 level of the spinal cord. The balloon was rapidly inflated with 15 μ l of saline for 5 min using a precise sampling device. Subsequently, the balloon was deflated and removed, and the wound was quickly sutured in surgical layers. The injury produced

by inflation to 15 μ l for 5 min caused complete paraplegia for 2–3 days after injury, followed by gradual recovery from around 4 weeks after SCI.

Temperature manipulation

After injury, animals were randomly divided into normothermic (A, $n = 15$) and hyperthermic (B, $n = 15$) groups. Halothane anaesthesia (1.0–1.5%) was maintained for an additional 3.5–4 h after injury. During this period, the animals were wrapped in a thermal blanket and placed in a thermoisolating plastic box. In the hyperthermic group, the thermal blanket was heated to 41–42°C. The rectal temperature increased to the desired 40°C within 30–45 min of heating. After reaching this point, body temperature was maintained at $40.5 \pm 0.5^\circ\text{C}$ for a fixed period of 3 h. Animals in the normothermic group underwent exactly the same procedure, but their rectal temperature was maintained at $37 \pm 0.5^\circ\text{C}$ throughout the same time interval. After this period, the anaesthesia was disconnected, and the animals were placed in their cages. Their rectal temperature was further monitored for at least 30 min to document its spontaneous decrease. If needed, a heating lamp heated animals until their body temperature became stabilized at physiological levels (Figure 1).

Postoperative care

Animals were housed in pairs to reduce stress from isolation on a 12-h light/dark cycle with standard rat chow and water *ad libitum*.

Figure 1 Shows body temperature recordings during and after injury in a representative normothermic and hyperthermic animal, respectively. The rectal temperature of the hyperthermic rat increased to the desired 40°C within 30–45 min of heating. After reaching this point (denoted with an arrow), body temperature was maintained at $40.5 \pm 0.5^\circ\text{C}$ for a fixed period of 3 h. Animals in the normothermic group underwent exactly the same procedure, but their rectal temperature was maintained at $37 \pm 0.5^\circ\text{C}$ throughout the same time interval

After SCI, manual bladder expression was performed. Generally, the expression of the bladder was performed twice a day during the early postoperative period; with the improvement of the animals' condition, it was performed once a day until the end of the second week, by which time a reflex bladder was established. The rats were carefully monitored for evidence of urinary tract infection or any other sign of systemic disease.

Neurological outcome evaluation

All animals were allowed to survive for 4 weeks after SCI. Their locomotor function was evaluated in an open field test, performed first at 24 h prior to SCI then 24 h after injury, and then once a week throughout their survival period. Movements of their hind limbs during locomotion were quantified using a BBB scale ranging from 0 to 21, where 0 reflects no locomotor activity and 21 reflects a normal performance.²⁴ The rats were tested on varnish surface of the table and the circular arena (90 cm in diameter) was a border with 20 cm height paperboard wall. Each rat was tested for 4 min with two examiners blinded to the treatment status observing both fore- and hindlimb movements.

As a measure of overall health, the body weights of animals were recorded before each testing procedure.

Histopathological outcome evaluation

At 28 days postinjury, the animals were deeply anaesthetized with ketamine/xylazine, their chest was opened and transcardiac perfusion was performed with physiological saline followed by 4% paraformaldehyde in phosphate buffer. The entire spinal cord was left in spinal channel overnight and then removed and post-fixed in the same fixative for at least 1 week.

A 2 cm long segment of the spinal cord containing the lesioned site was dissected and embedded in paraffin. The whole segment was serially cut, and a series of 20 sections (thickness 5 μ m) was collected (1 mm distance between individual sections). The sections were stained with Luxol Fast Blue and Cresyl Violet to facilitate discriminating between grey and white matter at low magnifications. Every section was imaged using a digital camera; high-resolution images were used to delineate the spared white and grey matter, and their areas were measured using image analysis software (Image Tool for Windows 2.00). For statistical analysis, 13 lesion-centred sections were used from each spinal cord. The volume of the spared tissue in this 11 mm long segment was calculated as the sum of cross-sectional areas multiplied by the distance between them.

Data analysis

In individual animals, BBB scores were averaged across hind limbs, and intergroup differences analysed by using the nonparametric Kruskal–Wallis and Mann–Whitney *U*-tests. Morphometric measurements were used to construct plots of consecutive cross-sectional areas of

the spared tissue at individual levels of the spinal cord rostral and caudal to the epicenter. The differences at each level were analysed using the Kruskal–Wallis and Mann–Whitney *U*-tests. Body weights at individual survival time points and the calculated volumes of the spared tissue within the 11 mm long segments were compared by the unpaired Student's *t*-test. All values are expressed as means $\pm$ SEM. Differences between groups were considered statistically significant if $P < 0.05$.

Results

Overall recovery

Transient hyperthermia had no obvious effects on the general recovery of the paraplegic animals. On the first day after SCI, all rats were completely paraplegic, and their bladders had to be emptied manually for at least 2 weeks postinjury. In accordance with our experience in previous studies, during the first week, the animals lost approximately 10% of their body weight. However, during the second week, they started to gain weight, indicating good overall health. No intergroup differences in body weights were found during the survival period (Figure 2). Mortality was similar in both groups. Three and two animals died prematurely in the normothermic and hyperthermic groups, respectively. All of these premature deaths were caused by urinary complications.

Behavioural outcomes

Locomotor performance, expressed as BBB scores collected from five consecutive testings, is shown in Figure 3. At 24 h after SCI, all animals were completely paraplegic in both groups. A gradual improvement in

Figure 2 Effect of hyperthermic treatment on body weights. No statistically significant differences were observed ($P < 0.05$)

Figure 3 Recovery of hindlimb function over time after SCI in group B ($n = 12$, $\circ$ -) that received hyperthermic treatment (40 min after SCI for 3 h at 40–41°C) and group A ($n = 14$, $\bullet$ -) that received normothermic treatment (40 min after SCI for 3 h at 37°C). Locomotor function graded on an expanded scale (BBB) that ranges from 21 in normal rats to 0 in rats with complete hindlimb paraplegia. Data are presented as means $\pm$ SEM. *denotes statistical differences ($P < 0.05$)

their neurological recovery could be observed throughout the 4-week survival period. Beginning from day 7, scores in the normothermic group were better than those in the hyperthermic one, and the differences reached statistical significance at 21 and 28 days postinjury.

Histopathological outcomes

Morphometric measurements of the cross-sectional areas of spared tissue are summarized in Figure 4. In the hyperthermic group, most of the values were lower than those in the normothermic group. A similar tendency was observed in both grey and white matter, but the differences did not reach statistical significance. Similarly, calculations of the volumes in 11 mm long segments showed that the volumes of spared grey and white matter were larger in the normothermic group, but again, the differences were not statistically significant (Figure 5).

Discussion

Results of the present study demonstrate that transient post-traumatic hyperthermia, lasting for 3 h, aggravates behavioural outcome after compressive SCI in rats. The detrimental effect detected in our experiment is a consistent, significant, and long-lasting worsening of BBB scores used to quantitate the degree of spontaneous locomotion in hyperthermic animals.

As far as we know, there is only one paper studying the effect of hyperthermia on SCI. Yu *et al*²⁵ described that hyperthermia worsened outcome parameters after

Figure 4 Effect of hyperthermic treatment on lesion morphometry at 4 weeks after SCI. Data points represent the area of spared WM (2) and GM (1) in spinal cord sections 5 mm cranial and 6 mm cranial and caudal to the injury epicenter (means $\pm$ SEM). Intergroup differences are statistically non-significant

Figure 5 Effect of hyperthermia on spinal cord tissue volume. Statistical analysis showed no significant difference between hyperthermia-treated and control groups

SCI. They studied the influence of hyperthermia on behavioural parameters and area of tissue damage after SCI. Our goal in this study was to distinguish whether hyperthermia influences more white or grey matter after SCI. Histopathological analyses revealed that hyperthermia increased tissue loss in both white and grey matter. These differences, however, did not reach statistical significance. It should be noted that in the present study, the extent of injury in both groups was unexpectedly large. We have analysed 13-mm long segment of the lesioned spinal cord, but the results indicate that our morphometry did not involve the whole lesion, which apparently continued in both cranial and caudal directions beyond the analysed segment. This large extent of the lesion in both groups was rather surprising given our experience with this model.^{22,23,26}

The only difference compared to our previous experiments was that all control animals underwent prolonged halothane anaesthesia during which their body temperature was maintained at normo- or hyperthermia. We can therefore speculate that the worsening was caused by (i) some detrimental effects of anaesthesia, or (ii) by strictly maintaining body temperature at 37°C. We have no data on blood pressure changes but it is known that halothane anaesthesia lowers blood pressure and this might adversely affect perfusion in the damaged tissue. We routinely do not monitor body temperature after finishing acute experiment and disconnecting animal from anaesthesia. However, in models of severe SCI, spontaneous drop in body temperature has been observed in paraplegic animals.^{27,28} This spontaneous hypothermia might provide some degree of 'natural' hypothermic protection. If this was the case with our model as well, it is possible that animals maintained normothermic for 3 h after SCI were effectively hyperthermic compared to unregulated animals in our previous studies.

Prolonged systemic hyperthermia might have influenced also other organs with impact on vital functions in paraplegic animals. However, mortality, body weight changes and overall recovery was similar in both experimental groups, indicating that the negative effect of hyperthermia was specific to nervous system.

The intergroup difference between normothermic and hyperthermic animals was unambiguous and clearly documents that relatively short-lasting body temperature changes can significantly modulate long-term outcomes after SCI.

Detrimental effect of hyperthermia on TBI is well-documented,^{13,29} and rigorous control of normal body temperature is the current standard of care for the brain-injured patients.³⁰ In contrast to this, in SCI, the relationship between body temperature and the outcome has not been systematically studied. One obvious reason is that for demonstration of small (but significant) effect in clinical situations, recruitment of very large groups of patients would be necessary, given the variability of SCIs.

It has been generally accepted that after SCI, tissue damage develops as a two-step process that evolves dynamically over time. The primary damage caused by mechanical disruption of tissue elements initiates a cascade of secondary events, which promote further destruction of the spinal cord. Secondary events include hypoperfusion, inflammatory processes, alterations of the biochemical environment, that is, excitotoxicity, release of cytokines, etc. Importance of individual events and their contribution to the final injury remains to be determined, but each of them represents a potential target for therapeutic intervention.

SCI is a specific case of CNS injury, as neurological consequences are almost exclusively due to the white matter damage, resulting in disconnection between the distal part of the spinal cord and supraspinal structures. With regard to this, the most important part of secondary processes is secondary destruction of interrupted nerve fibres in the vicinity of the primary lesion. Consecutive histological mapping indicates that the initial damage, which is located within the first 4 h mostly in the grey matter, extends progressively for at least 3 days after injury, and the final lesion involves greater part of the white matter, as well.³¹

Our experiment indicates that hyperthermia accelerates the processes causing secondary white matter injury *in vivo*. Anoxic damage to the white matter has been extensively studied *in vitro*.^{32,33} White matter has been considered relatively resistant to ischemia, as it tolerates periods of anoxia up to 30 min without permanent damage. One explanation for this relative resistance is the ability of axons to fuel their oxidative energy production from lactate. In the absence of glucose, lactate is produced by astrocytes from their glycogen stores, and axon function lasts until glycogen stores are exhausted.³⁴ However, prolonged anoxia results in irreversible injury, and hyperthermia dramatically worsens anoxic axonal injury *in vitro*.¹⁴

Temperature manipulations can modulate tissue metabolic and oxygen requirements, oedema formation, haemorrhage, and the stabilization of the cell membrane.³⁵ Temperature changes after SCI also modulate the release of excitatory amino acids,³⁶ leucocyte accumulation,³⁷ lipid peroxidation and the frequency of TUNEL-positive cells.³⁸ Michelfelder and Milde³⁹ demonstrated that hypothermia reduces both cerebral and spinal cord metabolic rates for oxygen equally, at approximately 5% per centigrade degree. Dietrich *et al* demonstrated that myelinated axons were very sensitive to post-traumatic brain hyperthermia. Stys *et al*³³ reported that after ischemia of the white matter, the intracellular accumulation of Ca^{2+} by way of the reverse operation of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger occurred. The accumulation of Ca^{2+} activates various lysoytic and proteolytic enzyme systems, which, if the activation is severe and prolonged, lead to irreversible cell injury. Since the rate of Ca^{2+} movement by this exchanger is highly temperature sensitive, it is possible that hyperthermia worsens neurological outcome via increased Ca^{2+} accumulation. Hyperthermia also induced the

immunoreactivity of dynorphin A, which has the capacity to induce cell injury via mechanisms involving NO formation.^{40,41}

In summary, our results demonstrate that transient post-traumatic hyperthermia can aggravate functional outcome after SCI. This indicates that, similar to patients after stroke and TBI, after SCI body temperature should be continuously monitored, effective and immediate steps should be taken to prevent occurrence of hyperthermic episodes in these patients.

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