REVIEW

Acta Neurobiol Exp 2020, 80: 172–178
DOI: 10.21307/ane-2020-016

Since 1928 Acta Neurobiologiæ Experimentalis

Hypothermia in the course of acute traumatic spinal cord injury

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In this review we briefly discuss animal experiments involving acute traumatic spinal cord injury (SCI) and the need for larger animals in testing experimental therapies. This literature overview, including the discussion of our own results from animal models, examines the use of hypothermia as a treatment method for SCI. Finally, we report the results of hypothermia application in clinical trials. Minipigs have been proposed as a potentially preferable model to rodents (typically rats) for predicting outcomes in human SCI due to their closer anatomical similarity to humans. In various animal studies, hypothermic treatment applied in the acute phase after SCI has resulted in neuroprotective effects, most likely due to inhibition of blood flow and oxygen consumption and reduction of overall metabolic activity and inflammation, resulting in improved nerve tissue sparing. Small-scale human clinical trials have been carried out, involving general (whole-body, systemic) or local hypothermia (close to the SCI site), with encouraging results. Nevertheless, further multi-center, randomized, double-blind studies with much larger patient numbers are necessary so that protocols can be standardized in order for hypothermia treatment to be reliably applied in clinical practice.

Key words: hypothermia, spinal cord injury, rats, pigs

INTRODUCTION

Traumatic spinal cord injury (SCI) is a life-threatening neurological condition resulting in partial or complete loss of motor, sensory and autonomic innervation below the injury level. The consequences of SCI include not only a decreased quality of life for the injured but also an adverse socio-economic impact on their relatives and society as whole (Piatt et al., 2016; Nori et al., 2017). Despite recent progress in the understanding of SCI pathophysiology and progress in experimental therapy, the results of clinical treatment strategies remain unsatisfactory (Ahuja et al., 2017). Current surgical practices allow for safe decompression and subsequent stabilization, which primarily benefits the surrounding structures and only slightly affects the secondary pathophysiology of the SCI itself (Ahuja et al., 2017; Rath and Balain, 2017). Additional pharmacological treatment primarily focuses on one or two mechanisms of spinal pathology, and thus it currently results in only minimal functional improvement. Other neuroprotective strategies such as therapeutic hypothermia alone or in combination with other interventions, whose mechanisms of action are multifactorial and may thereby protect nervous tissue and minimize overall loss of neurological function, are therefore being considered for experimental and clinical trials.

Experimental animals used in SCI studies

Although detailed descriptions of the causes and symptoms of traumatic SCI date back to ancient Egyptian times, intensive research into the pathological pro-

Received 12 April 2019, accepted 11 March 2020

cesses of SCI using experimental animals began only in the second half of last century. Initially, dogs, cats and monkeys were used (Bigelow and McBirnie, 1953). In these studies, spinal injury models varied and produced differing severities of injury. Furthermore, non-standardized histological, electrophysiological methods and functional measurements were used. Inconsistencies in experimental animal behavior assessment scales due to interspecies anatomical differences (dogs, cats, non-human primates) often resulted in contradictory findings. This led to the standardization of experimental procedures and the use of laboratory rodents. Rats have become the most commonly used laboratory animals due to several similarities with humans in the pathophysiology and recovery processes experienced after traumatic SCI (Rosenzweig and McDonald, 2004). Despite the indisputable benefits of rat experiments, there are certain limits that have to be considered before translating results from rodents into human practice. Thus, in many cases the promising treatment documented in rats failed when applied to humans (Rosenzweig and McDonald, 2004).

These findings suggest that the experimental therapy found to be beneficial in rats suffering from SCI should be tested on larger animals, such as minipigs and pigs, prior to introduction into human treatment (Kwon et al., 2015). Because the anatomical parameters of pig organs correlate with those in humans, they provide a more relevant platform for pharmacotherapy, biomaterial inventions, electrophysiological and rehabilitation studies (Bassols et al., 2014). Pigs are considered to be one of the most suitable animal species used in translational medical research. They share similar anatomical and physiological characteristics with humans, particularly the cardiovascular, urinary, reproductive and digestive systems (Bassols et al., 2014). In the pig spinal cord, the localization of the corticospinal tract (CST) inside the lateral columns of the white matter is more similar to humans than to rodents (Leonard et al., 2017). In terms of anatomical function, the CST plays a key role in locomotion of the pigs' hindlimbs. This is in contrast to rodents, where the rubrospinal tract is predominantly involved. These anatomical and functional similarities to humans provide support for pigs as a reliable animal model for preclinical research into traumatic SCI (Leonard et al., 2017). In general, it should be noted that both small and large animal models of SCI have limitations in their ability to predict outcomes in human SCI (Rosenzweig and McDonald, 2004; Bassols et al., 2014).

Spinal cord injury pathology

Extensive research based on animal models has aided in the clarification of complex nervous tissue processes that occur in the spinal cord after injury. The pathophysiological process induced by tissue damage is divided into two phases – the primary injury and the secondary injury. Primary injuries arise from mechanical disruption caused by compression, contusion, transection or distraction/stretching of the spinal column (Rosenzweig and McDonald, 2004; Ramer et al., 2005; Wang and Pearse, 2015). This injury usually occurs with vertebral bone fracture and/or dislocation of the spine. However, spinal epidural hematomas or abscesses may cause spinal cord compression and injury as well. Within minutes of the mechanical insult, the secondary mechanisms of SCI begin, accompanied by subsequent pathophysiological and molecular changes that amplify the injury and enlarge the lesion site. Currently, there are several well-explored secondary mechanisms of SCI (Ramer et al., 2005), negatively affecting not only the cells that survive the primary damage but also the surrounding nerve tissue, thus extending the primary damage (Ramer et al., 2005) (Table I). Subsequent findings and an increased understanding of these processes provided opportunities to actively intervene during their course of development in order to minimize their unfavorable progress (Ramer et al., 2005). The purpose of neuroprotective treatment is to reduce the secondary damage that typically follows a primary insult and increases its extent (Ahmad et al., 2014).

Hypothermia-induced neuroprotection

Hypothermia has been used for therapeutic purposes for hundreds of years. Application of snow and ice to wounded areas of the body was recommended by Hippocrates (Chang et al., 2007). However, in 1700 the Scottish physician James Currie was the first to successfully use whole-body cooling for the treatment of various clinical disorders, and he documented the range of human body temperatures in health, disease and experimental conditions (Karnatovskaia et al., 2014). Since 1953, when Bigelow detected the positive effect of hypothermia on the brain during heart surgery, the systematic study of its neuroprotective effect on nerve tissue has advanced (Bigelow and McBirnie, 1953).

Hypothermia-mediated neuroprotection has been reported for all phases of nerve tissue damage, but the greatest beneficial effects have been observed during the first stages of injury (Table I). In acute SCI (within 48 h of injury), hypothermia reduces blood flow, oxygen consumption and total metabolic activity in the tissue, oxidative stress and the formation of free radicals are reduced and inhibition of lactate formation prevents the development of acidosis. These factors lead to decreased edema formation. Concomitantly, with the re-

Table I. Summary of pathophysiological events during SCI.

Time sequence	Phase of injury	Ongoing processes caused by mechanical damage: compression, contusion, transection	Therapeutic goals
		Primary	
≤ 2 hours		blood-brain barrier damage; vasospasm; decreased blood flow; hemorrhage; edema; ischemia; spinal shock; microglia activation; neuronal death	- surgical decompression: relieving mechanical pressure on the vascular circulation, reducing hypoxia and ischemia - spinal cord stabilization - neuroprotection mediated by glucocorticoid application
		Secondary	
≤ 48 hours	Acute	microglia activation; neutrophil infiltration; release of pro-inflammatory cytokines: IL-1β, TNFα, IL-6; chemokines: CXCL1, CXCL12; oxidative stress: increased ROS production NO, iNOS; lipid peroxidation; glutamate excitotoxicity; ionic imbalance; mitochondrial damage; apoptosis of neurons and oligodendroglia; demyelination; blood-brain barrier damage; neuronal cell death; axonal swelling; hypotension; hypoxia	- neuroprotection mediated by combined hypothermia and glucocorticoid application - immuno-modulation - remyelination mediated by delivery of oligodendroglial cells
≤ 14 days	Subacute	infiltration of monocytes, macrophages, microglia; reactive astrocytosis; apoptosis of oligodendrocytes; demyelination; cyst and glial scar formation; neuropathic pain; chemokine release; phagocytosis; blood-brain barrier recovery; edema regression	- neuroprotection mediated by glucocorticoid application - immuno-modulation - scar degradation
≤ 6 months	Transient	glial scar and cysts maturation; apoptosis of oligodendrocytes; demyelination; lesion stabilization; release of neurite growth inhibitors: Nogo, MAG, ROCK; Wallerian degeneration	- rehabilitation: body weight-supported gait training
≥ 6 months	Chronic	neuro-reparatory processes; anti-inflammatory phenotype of microglia and macrophages (M2); Wallerian degeneration	- rehabilitation: robotic rehabilitation of SCI individual

duction in blood supply to the traumatized neuronal tissue, there is also reduced lymphatic drainage. On the other hand, the decreasing lymph flow caused by hypothermia subsequently results in the accumulation of metabolic waste, which needs to be removed during the following rewarming period (Chen and Chien, 1977; Topuz et al., 2010). In the subacute phase (up to 2 weeks after injury), hypothermia induces an inhibitory effect on the inflammatory process, as well as protecting the blood-brain barrier (Karnatovskaia et al., 2014) (Table I).

During deep cooling, i.e. below 25°C, the most commonly observed effect was on oxygen consumption, while during less intense cooling the secondary changes in the damaged tissue were predominantly affected (Strauch et al., 2004; Yoshitake et al., 2007; Dietrich et al., 2011).

General hypothermia

General (systemic) hypothermia can be induced by physical and pharmacological means. Methods for inducing physical hypothermia include a cooling blanket or vest, ice pad or infusion of rapidly-cooled saline (Ikeda et al., 2012) (Table II). Pharmacological hypothermia is related to several classes of drugs: cannabinoids, opioid receptor activators, transient receptor potential vanilloid, neurotensins, thyroxine derivatives, dopamine receptor activators, hypothermia-inducing gases and others (Table II). However, both the neuroprotective action and side effects of each must be considered (Zhang et al., 2013).

Most SCI experiments have been performed at the level of the thoracic and lumbar segments of the spinal cord, while the number of experiments conducted in the cervical area in rats is significantly lower (Jou, 2000; Strain and Waldrop, 2005; Lo et al., 2009). The process of cooling is primarily carried out at 30-34°C body temperature, lasting from 20 min to 4 h or until the end point of the experiment. Hypothermia is induced immediately or up to 15-30 min after induction of SCI (Yu et al., 2000; Westergren et al., 2001). Maybhate and colleagues (2012) applied modest systemic hypothermia of 32 ± 0.5°C lasting for 2 h after thoracic SCI in rats.

Their results revealed significant improvement in electrophysiological, functional neurological and histological findings.

Our study also demonstrated beneficial effects of systemic hypothermia. Rats underwent SCI at the thoracic level (Th8-Th9), followed by application of ice pads, which induced systemic hypothermia at 32°C with gradual rewarming to 37°C and resulted in improved locomotion as well as bowel functions (Grulova et al., 2013). Significant bladder function improvement was

recorded after five days, while complete bladder recovery was observed two weeks after treatment (Grulova et al., 2013). This was significantly better compared with the experimental group without systemic cooling, in which the bladder recovery took as much as 25-28 days, confirming that hypothermia induced neuroprotection of bladder function. Furthermore, histological analyses revealed enhanced sparing of spinal cord tissue with smaller cavities and more preserved white and gray matter (Grulova et al., 2013).

Overall, the most effective systemic hypothermia in relation to other organs is when moderate cooling between 32-34°C is induced immediately after the trauma. Hypothermia should last up to 48 h, and should be followed by slow rewarming at 0.1°C per h (Martirosyan et al., 2017). A recent study has provided evidence that systemic hypothermia (28°C) can effectively counteract the increase in extracellular ascorbate concentration after acute SCI. Thus, a significant reduction in spinal cord ascorbate concentration in rats following spinal injury may be related to neuroprotective mechanisms during the secondary phase of injury (Zhang et al., 2019).

The drawback of systemic hypothermia correlates with the reduction in total body temperature, which influences all biological processes in the body. Each of the therapeutic phases of hypothermia, induction and maintenance as well as return to normal body temperature, is associated with risks, especially to the cardiorespiratory apparatus (Karnatovskaia et al., 2014). However, after additional and more detailed experiments, it is expected that the clinical application of general hypothermia will gradually increase in the near future.

Local hypothermia

Another cooling procedure uses local hypothermia, in which surgical and decompression procedures allow the spinal cord surface to be accessible for cooling. The principle of local spinal cord hypothermia is to cool the nerve tissue near the epicenter of the damage. Several local cooling systems have been developed for SCI (Bazley et al., 2014). The cooling process is carried out either intrathecally or epidurally, in which opening of the dura mater (durotomy) would be necessary or not, respectively, to cool the spinal cord directly. Transcutaneous cooling is another possible mechanism for decreasing paraspinal temperature that avoids laminectomy (Demian et al., 1971; Hansebout and Hansebout, 2014) (Table II). The most frequently utilized technique involves heat exchanger tubing being inserted under the targeted site of spinal injury (paravertebral muscle in thoracic segments) and by circulating cold solution local hypothermia is achieved (Table II). In several studies, a heat exchanger (M- or U-shaped copper tube) was subcutaneously inserted above the spinal column at the lesion site in the thoracic area (T6-T8) spinal hypothermia and was induced at 30 (0.5)°C for either 5 or 8 h, followed by gradual rewarming to 37°C. This approach improved motor behavior recovery for a long period, lasting up to 8 weeks (Teh et al., 2018). In another study, epidural perfusion was used in rats after SCI to induce local hypothermia (18°C) for 170 min with gradual rewarming to 37°C. Several beneficial effects were observed in rats subjected to hypothermia: downregulation of axon regeneration inhibitors (RhoA, ROCK-II, NG2, Neurocan, Brevican, and Nogo-A), decreased demyelination and enhanced axon regeneration, which ultimately resulted in recovery of hindlimb function (Xu et al., 2016). After the observation of promising results in rats, it was necessary to verify the effects of hypothermia in larger animal models (Kwon et al., 2015).

Initially, studies were proposed for the prevention of paraplegia associated with thoracoabdominal aneurysm repair (Coselli et al., 2002). These experiments confirmed that epidural cooling provided by a special catheter containing cold saline solution circulating in an isolated lumen induces a protection effect against ischemic SCI in pigs (Mori et al., 2005; Yoshitake et al., 2007). Based on these encouraging results, further spinal cord cooling methods have been developed and verified in other SCI models.

In our previous experiment, we studied the effects of local hypothermia on selected parameters in a minipig model of contusion SCI using a computerized compression device. In this model we were able to scale the impact strength (8N, 15N, 18N) and thus compare the findings for varied degrees of contusion (Gedrova et al., 2018; Zavodska et al., 2018). In the control (normothermic) group, the graded lesion at the impact site was recorded as 47% loss of tissue with a force of 8N impact, 67% loss of tissue with a force of 15N impact and 79% loss of tissue with a force of 18N impact after 9 weeks of survival. Moderate injury impact (8N and 15N) accompanied by local epidural cooling with 19°C, induced 30 min after SCI for 5 h, revealed an increase of 11% in the gray mass at the site of injury. Nerve tissue sparing was also observed in the white matter, especially in the dorsal columns (with 13-15% sparing). While hypothermia after the injury induced by an impact of 8N led to greater functional improvement, after the more severe impact of an 15N impact it had no beneficial effect on locomotion. These findings raised the question of the extent to which nerve tissue sparing must be present in order to achieve a better functional outcome. We therefore systematically examined changes in the cranial and caudal segments (3 cm long) adjacent to the central lesion. Local hypothermia after 8N force impact significantly

increased the number of fibers and neurofilaments in the gray and white mass near the epicenter. The number of nerve fibers (axons) in the *funiculus lateralis* was increased in the cranial segment (+1 cm) by 25% and in the caudal segment (-1 cm) by 19%, which correlated with faster functional improvement in the hypothermia-induced group compared to the normothermic control. Thus, the preserved integrity of the nerve tissue in the cranial as well as the caudal segment adjacent to the epicenter with corresponding neurofilaments in the lateral columns may be critical for recovering motor function. In contrast, local hypothermia after the 18N force impact, despite detected neuroprotection in white and gray mass of the caudal segment, ultimately did not lead to improvement in the final neurological score. It is assumed, therefore, that the 18N force is a very strong impact causing serious pathophysiological changes against which hypothermia is ineffective.

Therapeutic hypothermia used in human clinical trials

The positive effects of local and general hypothermia in animal experiments following acute traumatic SCI has led to clinical trials. Among the first was a clinical trial of local hypothermia in patients with spinal epidural abscess (Jackson and Assam, 1964). Further advances in inducing and maintaining hypothermia have enabled more accurate evaluations of this form of treatment. Demian and colleagues (1971) demonstrated its benefit in three patients with acute SCI in a cervical segment, without change in their overall body temperature, blood flow and respiratory parameters. This led to the launch of clinical studies including larger numbers of patients. Romodanov and colleagues (1979) performed hypothermia in 113 patients, and they observed its positive effects in reducing bleeding and swelling of the spinal cord, diminishing muscle spasticity, improving motor function and alleviating pain. Hansebout and Hansebout (2014) examined the effect of local hypothermia in a study of 20 patients after SCI in the cervical and thoracic region. After decompression of the spinal canal, which was performed within 8 h of injury, they cooled the intact dura for 4 h until the temperature of the dura was 6°C. They observed an improvement in mean motor and sensitivity scores. These patients, treated with surgical decompression, glucocorticoid administration and regional hypothermia, experienced better recovery than might have been expected with standard treatment procedures (Hansebout and Hansebout, 2014). Together with ongoing local hypothermia testing, clinical trials using systemic hypothermia have also been launched. The case report of an NFL player in the USA, who suffered a C3/4 dislocation fracture causing complete motor paralysis and sensory loss (ASIA A grade), has been informative in terms of a more systematic study. Timely systemic hypothermia (33.5°C) was performed for a period of 36 h along with surgical decompression and methyl prednisone administration. Afterwards, along with rehabilitation, rapid neurological improvement and ASIA conversion from A to D grade was observed (Cappuccino et al., 2010). In another study, Levi and colleagues (2009) demonstrated the safety of systemic hypothermia using a catheter, which was then endovascularly inserted into patients with SCI (Levi et al., 2010). Similarly, Madhavan and colleagues (2012) and Dididze and colleagues (2013), noticed significant overall improvement after using systemic intravascular hypothermia (33°C), induced immediately post-surgery and maintained for 48 h post-operatively. This management paradigm failed to counteract deterioration in respiratory complications, with throm-

Table II. Cooling methods to induce and maintain hypothermia.

General Hypothermia			Local Hypothermia
Physical Methods		Pharmacological Methods	Physical Methods
Surface cooling devices	Endovascular cooling	Drugs	Heat exchanger tubing system
air and water circulating cooling blankets; hydrogel-coated water-circulating pad; ice packs, pads; wrapping garments; cooling-vest	intravenous infusion of: ice-cold saline, ice-cold Ringer's solution	cannabinoids; opioid receptor activators; transient receptor potential vanilloid; neurotensins; dopamine receptor activators; adenosine; inhalational anesthetics	intrathecal; epidural; paraspinal; transcutaneous

Cannabinoids: bind to their specific CB1 receptors distributed in the central nervous system, particularly of preoptic anterior nucleus of the hypothalamus (POAH), inducing hypothermic effects.
Opioids: induce hypothermia *via* interaction with opioid receptors-μ, κ, δ.
Transient receptor potential vanilloid 1: nonselective cation channel that plays a thermoregulatory role through peripheral sensors and the POAH.
Neurotensins: tridecapeptide neurotransmitter mediating hypothermia by a G protein coupled receptor – NT receptor type 1 (NTS1).
Dopamine receptor activators: induce hypothermia *via* D2-like receptors.
Adenosine: triggers hypothermia *via* agonism at A1 and A3 adenosine receptors.
Inhalational anesthetics: sevoflurane, isoflurane induce hypothermia by inhibiting central thermoregulation.

boembolic complications occurring in 14.2% of patients (Dididze et al., 2013).

With this in mind, a clinical trial with systemic hypothermia following acute SCI is currently under way. In 2017, the Miami Project to Cure Paralysis initiated a larger phase II/III trial of systemic hypothermia in acute (≤ 24 h) cervical SCI (ASIA C) patients. In this prospective multicenter case-controlled study, patients receive modest (33°F) intravascular hypothermia for 48 h (Donovan and Kirshblum, 2018). Another clinical trial with systemic hypothermia was completed in January 2019, the first results of which are expected in 2020. Nevertheless, the limited number of patients in these studies with differently applied hypothermia, time of initiation and time of duration does not yet allow for its application in clinical practice, and it remains at the clinical trial stage. A multi-center, randomized, double-blind study with a significantly greater number of patients and a clearly defined method, timing and duration of administration, temperature range and technical execution will be required.

CONCLUSIONS

Hypothermia, as part of acute traumatic SCI treatment, has shown promising neuroprotective effect in experimental and preclinical animal models. However, its clinical application is still very limited due to several factors. Systemic hypothermia is associated with adverse effects on other organs, especially the cardiorespiratory apparatus. Local hypothermia is time delayed and requires advanced surgical procedure. Large clinical trials are therefore required for investigation and standardization of protocols providing the most beneficial effect of hypothermia for treating SCI. In particular, identification and extension of the therapeutic window and determination of the effective duration of hypothermia but also optimal warming procedures are at the forefront of current medical research. The neuroprotective effect and the availability and affordable cost of the combined treatment, which is necessary for this type of SCI damage, create significant interest for further experimental and, particularly, clinical studies in hypothermia.

ACKNOWLEDGMENTS

The work was supported by research grants ERANET-AxonRepair, APVV 15-0613, APVV 19-0193, VEGA 1/0571/17, VEGA 1/0376/20, IGA UVLF 02/2019: "Stratification of patients with canine cognitive dysfunction, application of innovative stem cell therapy."

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