Epidural perfusion cooling protection against protracted spinal cord ischemia in rabbits

IVO VANICKÝ, D.V.M., MARTIN MARŠALA, M.D., JÁN GÁLIK, AND JOZEF MARŠALA, M.D.

Institute of Neurobiology, Slovak Academy of Sciences, Košice, Slovak Republic

The protective effect of a modified epidural cooling technique was assessed in a rabbit spinal cord ischemia model. The epidural space around the lumbar segments with induced ischemia was continually perfused with cold (5°C) isotonic saline via two communicating spinal canal openings. This procedure allowed the spinal cord to be kept deeply hypothermic (< 15°C within central gray matter) during the ischemic period.

The animals were subjected to either normothermic ischemia (Group A) or hypothermic ischemia (Group B). Each group contained three subgroups of animals undergoing 20, 40, or 60 minutes of aortic ligation. Their neurological outcomes were evaluated up to 48 hours posts ischemia, and the intergroup differences were compared. Two days posts ischemia, all of the animals were sacrificed by transcardial perfusion-fixation and their lumbar segments were processed for histopathological examination. In addition, in animals with 60-minute ischemia, spinal somatosensory evoked potentials were recorded during surgical intervention and again after 48 hours.

In the normothermic animals, a high incidence of paraplegia was detected: in 40% after 20 minutes of ischemia, in 75% after 40 minutes, and in 100% after 60 minutes. In contrast, all of the hypothermic animals exhibited full neurological recovery even after 60 minutes of ischemia. Both electrophysiological and histological observations clearly correlated with the neurological findings. The results suggest that deep spinal cord hypothermia produced by epidural perfusion cooling provides effective protection against protracted spinal cord ischemia in rabbits.

**KEY WORDS**  •  spinal cord  •  ischemia  •  hypothermia  •  epidural cooling  •  rabbit

ISCHEMIC spinal cord injury represents the main complication in surgical repair of thoracoabdominal aneurysms. Temporary aortic occlusion produces profound physiological changes in the organism, including critical reduction in spinal cord perfusion with a risk of irreversible injury. To increase spinal cord perfusion pressure, a number of techniques including left heart bypass, shunting, or cerebrospinal fluid (CSF) drainage have been developed, but their ability to prevent a relatively high incidence of paraplegia is limited. Similarly, the protective effects of various pharmacological agents are of minor clinical importance, especially after prolonged periods of ischemia. Induced hypothermia, either systemic or local, seems to provide the most potent protection against ischemia-reperfusion neural injury; however, cooling a relatively long spinal cord segment is technically difficult. Although systemic hypothermia has proved highly neuroprotective, its use is limited by unwanted systemic side effects, especially cardiac disorders, which may even occur at a body temperature of about 32°C. Regional hypothermic perfusion with cold fluid applied to the vascular bed represents an alternative solution; however, in the case of aortic occlusion, the technical aspects of this intervention present some problems which may discourage its general use. In addition, intravascular procedures add considerably to clinical morbidity and mortality. Direct local spinal cord cooling, applied either sub- or epidurally, has commonly been used after traumatic spinal cord injury. Although the beneficial effects in this case are limited to secondary pathomechanisms, the highly effective spinal cord cooling achieved and the almost complete avoidance of systemic complications support a wide use of this technique.

Extravascular perfusion cooling in spinal cord ischemia was reported by Negrin, who used a heat-exchange device to induce deep hypothermia and observed complete neurological recovery after 90 minutes of aortic cross-clamping in dogs. Similarly, we recently tested the efficacy of a simple cold fluid infusion into the epidural space in a canine model, and a protective
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Table 1: Neurological outcome at 48 hours postischemia in six groups of rabbits

<table>
<thead>
<tr>
<th>Neurological Outcome</th>
<th>20-Min Ischemia</th>
<th>40-Min Ischemia</th>
<th>60-Min Ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>no. of rabbits</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>outcome</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>2</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>paretic</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>paralyzed</td>
<td>2</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

*Group A = normothermic animals; Group B = hypothermic animals.
† One rabbit died before scoring.

The effect of moderate hypothermia (about 28°C) was also confirmed. In the present study of rabbits, we perfused the epidural space of the spinal canal between L-5 and T-11 so that most of the infused cooling fluid was allowed to flow out extracorporally. The hypothermia achieved by this technique during ischemia is below 15°C. The purpose of the present study was to test the applicability of this method and its protective effect in protracted spinal cord ischemia.

Materials and Methods

We used a modified rabbit spinal cord ischemia model. Experiments were performed on 26 rabbits of both sexes, each weighing 2.0 to 3.5 kg. The rabbits were separated equally into a normothermic (Group A) or a hypothermic (Group B) ischemia group. Each group contained three subgroups in which the animals were subjected to 20, 40, or 60 minutes of ischemia (Table 1).

Surgical Procedure

The animals were anesthetized with pentobarbital (30 mg/kg, intravenously). A small subcostal incision was made on the left side, exposing the abdominal aorta at the level of the renal arteries. A snare ligature was placed around the aorta distal to the left renal artery so that ischemia could be induced by tightening the ligature. After the ischemic period, the ligature was released and restoration of the blood flow was verified visually. The laparotomy was then sutured and the rabbits were returned to their cages.

In the hypothermic group, two midline incisions were performed under the L-5 and T-11–12 vertebrae, respectively. The musculature was removed at the lumbar level and a small incision was made in the intervertebral ligaments. A cannula (outer diameter 0.8 mm) was inserted into the epidural space for about 1 cm cranially, then fixed with dental cement to the L-6 vertebral laminae (Fig. 1). At the thoracic level, a partial laminectomy was performed carefully and the intervertebral ligaments were removed so that the epidural space was left widely open. Cold isotonic saline (5°C) was then infused via a cannula into the spinal canal. At the beginning of perfusion, the stream of saline usually

Fig. 1. Schematic drawing showing the epidural perfusion technique. A catheter (0.8 mm in outer diameter) is inserted into the epidural space and fixed with dental cement to the L-6 vertebral lamina. Most of the infused saline solution flows out via the T-11 laminectomy.

Neurological Examination

The animals were examined neurologically 24 and 48 hours postischemia. Neurological outcome was evaluated by recovery of motor functions, and was classified as normal (0), paretic (1), or paralyzed (2). The rabbits were considered to have normal motor function if they were able to hop with no signs of spasticity. Paretic animals could move about but had spastic hindlimbs and were unable to hop. Finally, the absence of muscle tone or contraction was defined as complete paralysis. In the paraplegic animals, Credé's maneuver was used to empty the bladder twice daily.

Spinal somatosensory evoked potentials (SSEP's) were recorded in both groups in the animals undergoing 60 minutes of ischemia. Their left sciatic nerve was exposed and stimulated with bipolar J-shaped electrodes. Two silver-wire recording electrodes were placed into small holes drilled into the L-6 and L-5 vertebrae. The recording electrodes were fixed to the bone with dental cement and left in situ during the survival period. Stimulation parameters included a 0.1-msec pulse duration and a 2- to 3-V charge at a rate of 4.1 Hz. The responses were amplified 30,000 times and processed through a high-pass filter (5-kHz) filter. Each recording represented an average of 16 repetition signals using PC-AT as a signal averager. The SSEP's were recorded before, during, and 30 minutes after ischemia, and the recording was repeated 48 hours postischemia.

At 48 hours postischemia, the animals were deeply anesthetized with pentobarbital (40 mg/kg) and trans-
cardially perfused with saline followed by 10% neutral Formol. The spinal cord was removed 24 hours later, immersed in the same fixative, and postfixed for about 14 days. The lumbar portion was dissected, and 20-μm frozen sections from each segment were either stained with toluidine blue or impregnated by the Nauta method.24

Results
In the normothermic animals, body temperature did not change during ischemia. The rectal temperature of hypothermic animals dropped about 2°C during the cooling period in spite of external heating. The temperature further decreased with the onset of recirculation but returned to normal within 30 minutes postsischemia. The cooling solution that flowed out represented about 58% ± 9% of the infused volume, with the rest of the perfusion fluid resorbed intrasomatically.

Neurological Status
Evaluation of the neurological score after 48 hours postsischemia is shown in Table 1. Small differences were found between outcomes at 24 hours and 48 hours. One Group A animal with 20 minutes of ischemia developed paraplegia only after 24 hours, and one paraplegic animal in Group A with 60 minutes of ischemia died before the second evaluation. In the normothermic group, 40% of animals were paraplegic after 20 minutes of ischemia. This number rose to 75% and 100% after 40 and 60 minutes of ischemia, respectively.

All of the hypothermic animals exhibited full neurological recovery, independent of the duration of ischemia. Early posts ischemic observations revealed that the hypothermic animals had well-preserved muscle tone of their hindlimbs even when recovering from anesthesia; however, slight paesis was present within the early period. The neurological status of the hypothermic animals evaluated at 24 hours and 48 hours was virtually indistinguishable from the control group with no ischemia.

Spinal Somatosensory Evoked Potentials
A representative pres ischemic SSEP recording from a normothermic rabbit is shown in Fig. 2A. The spinal cord potential complex consisted of typical deflections described as two positive waves, P1 and P2, and four negative waves, N1, N2, N3, and N4. In normothermic animals, the SSEPs became smaller with the onset of ischemia, and their latency increased. The most resistant N1 and N2 waves had disappeared within 21 ± 4 minutes in all animals. Within 30 minutes postsischemia, the N1 and N2 waves recovered completely, but late components were reduced to a single N3 wave, with its amplitude reaching 27% ± 7% of its pres ischemic value. After 48 hours, the late components were lost and the persisting N1 and N2 waves showed a slight decrease in amplitude (Fig. 2A).

In the hypothermic group, the onset of hypothermic perfusion caused a reduction in the amplitude of all components before ischemia; an absence of the response was observed after 15 ± 7 minutes of ischemia. All components became visible 30 minutes postsischemia, however, with deformed shape and increased latency. After 48 hours, the SSEPs resembled their pres ischemic patterns both in amplitude and latency of waves (Fig. 2B).

Histopathology
Group A: Normothermia. At 20 minutes of ischemia, the histopathological findings clearly correlated with the neurological status. The least-injured animals exhibited unaltered structure of gray matter. The paraplegic animals showed typical infarctions of variable size in the central gray matter, which were detected primarily in the medial zone spreading dorsally and ventrally, with predominant localization in the L4-6 segments. At 40 minutes, the animals developed wide infarctions and severe gliosis usually involving a greater part of the gray matter area. At 60 minutes, all animals exhibited large necroses with virtually complete loss of the neuronal pool. Histopathological processing with the floating technique was difficult due to disintegration of the tissue slices (Fig. 3).

Group B: Hypothermia. In all subgroups, the spinal cord structure was well preserved, although the Nauta method revealed occasional terminal-like degenerations in the neuropil. In addition, some neurons showed changes characterized as diffuse darkening of both cytoplasm and karyoplasm with poorly demarcated nucleus. The occurrence of such alterations seemed to correlate with the duration of ischemia, but no infarctions were detected in this group (Fig. 3).

Discussion
Despite all present efforts to ameliorate ischemic neuronal damage by pharmacological agents, hypothermia still draws the attention of many experimental laboratories.25 The special interest from a clinical point of view are several relatively recent studies showing the significant beneficial effects of mild hypother-
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Fig. 3. Representative photomicrographs of spinal cord segments from different animals at L-5. Left: Overall neuronal distribution. Toluidine blue, × 25. Right: Ventral horn details, in which some neurons exhibit altered cell body structure. Occasional terminal-like degenerations are present in the neuropil. Nauta impregnation technique, × 132. 1: Sections from a control animal. 2: Sections from a normothermic (Group A) animal with 60 minutes of ischemia and 48 hours of recirculation. Note the complete loss of neuronal pool and disintegrated gray matter. 3: Sections from a hypothermic (Group B) animal with 60 minutes of ischemia and 48 hours of recirculation.
Hypothermia (body temperature 32° to 34°C) and its usefulness even when applied after the ischemic event.3,19 However, in the case of spinal cord ischemia due to aortic cross-clamping, usually as a result of planned surgical interventions, all of the advantages of hypothermic protection (that is, all hypothermia induced before ischemia) support its general use. Systemic hypothermia carries a risk of cardiac disorders, thus precluding its routine clinical application. Ventricular fibrillation and cardiac standstill may appear at a body temperature of about 32°C, whereas central nervous system (CNS) tissue may tolerate 5°C without permanent neurological complications.25 This difference means that a large margin of low temperatures can be used if regional CNS cooling is technically available.

Regional hypothermic perfusion via the vascular bed has been shown to be protective for up to 45 minutes in a dog and pig model.10,19 The variability of the residual blood flow, however, can interfere with the cooling procedure and thus limit its efficacy. Impressive studies were published in the early 1970's by Negrin,5,26 who described extravascular perfusion for selective regional CNS cooling using a hypothermostat, a device developed for local cooling in neurosurgery. He reported protection against paralysis even after 90 minutes of ischemia induced with two thoracic clamps in dogs. The studies are lacking in extensive experimental groups and other neurological evaluations, but the effectiveness of deep hypothermia was recently confirmed by Berguer, et al.,1 who used a subarachnoid cold fluid-perfusion system. They reported recovery in 100% of dogs after double aortic cross-clamping for 45 minutes. In our laboratory, we have found a similar beneficial effect with simple epidural infusion of cold fluid inducing moderate spinal cord hypothermia in dogs.21 In the present study, we performed continuous perfusion of the epidural space by allowing most of the cooling fluid to flow out via the upper laminectomy. In pilot studies, we have found that this technique induces selective deep hypothermia (< 15°C) at a perfusion rate of 5 ml/min with only a slight effect on systemic temperature during ischemia (unpublished data).

The results of our experiments confirm the efficiency of this epidural cooling technique. All of the animals with induced hypothermia survived aortic ligation for up to 60 minutes without neurological sequelae, and the SSEP's revealed no abnormalities 2 days postischemia. We have considered a 48-hour period sufficient to detect postsischemic neurological consequences since the delayed onset of injury was negligible in longer ischemic periods (> 40 minutes). The minor histological findings involving occasional degeneration in the neuropil and the abnormal appearance of some cells without signs of disintegration can be attributed to either ischemic or hypothermic damage, or to a combination of both. We have no data concerning the dynamics of these alterations, and their character should be further studied in more detail. However, in vitro experiments showed that cultured spinal cord neurons can tolerate a body temperature of 17°C for 2 hours with no histologically detectable injury after rewarming to 37°C.20 To date, no optimal temperature for hypothermic protection against CNS ischemia has been determined. The level of hypothermia reached in our study was a result of our effort to induce as low a body temperature as possible rather than searching for optimal hypothermic conditions. A small decrease in systemic temperature at the onset of recirculation may add to spinal cord protection since postsischemic cooling proved to be effective in models of cerebral ischemia.32,6 This effect might be easily used as an adjunct by continuing hypothermic perfusion postischemia. However, we believe that hypothermia induced during ischemia provided the main protective effect which seemed to be efficacious in our experiment.

In spite of an increasing body of information, the pathophysiological mechanisms of ischemic neural injury are not fully understood. From a histopathological point of view, two basic types of injury, selective neuronal death and infarction, develop after CNS ischemia. Both types of injury were found in spinal cord ischemia models,13,12,21,22 indicating that similar mechanisms as those observed in the brain would be involved in ischemic spinal cord damage. Hypothermic protection, originally attributed to diminishing the metabolic demand before ischemia, becomes more complex in light of the latest investigations in this field. Leonov, et al.,14 have postulated a multifactorial mechanism coming into play in cerebral ischemia. Hypothermia quickly preserves high-energy phosphates; mitigates abnormal ion fluxes; reduces lactate production and tissue acidosis, free fatty acid production, and excitatory neurotransmitter release; slows enzymatic reactions; and protects the fluidity of lipoprotein membranes. All these factors may blunt a cascade of processes devastating the ischemic tissue.

In the present study, however, we have to consider another mechanism which may act in this case. Rabbit abdominal aorta ligation does not produce complete ischemia in the spinal cord. In most animals, about 2% of preischemic blood flow persists after 20 minutes of ligation,39 and hypothermia is known to raise regional blood flow in the spinal cord.31 Since we have no data about the spinal blood flow in our animals during hypothermia, it is possible that putatively enhanced residual blood flow becomes sufficient to cover low metabolism of the hypothermic tissue. In any case, recovery after extremely long periods of ischemia in this model indicates that the tolerable period of ischemia will extend even beyond this 60-minute period. We believe that epidural perfusion cooling represents an approach that should be re-evaluated in the clinical practice when the optimum perfusion technique and level of hypothermia are more exactly determined.

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