Significant advances in surgical techniques and protective maneuvers have resulted in a dramatic reduction in mortality associated with thoracoabdominal aortic replacement. However, spinal cord injury leading to paraplegia still occurs in a substantial number of patients. Reliable monitoring methods of spinal cord circulation are still required. Recently, we reported that continuous monitoring of cerebrospinal fluid (CSF) $P_O_2$ by a multisensor catheter (Paratrend; Biomedical Sensors, High Wycombe, United Kingdom) allows rapid detection of alterations in spinal cord circulation during experimental thoracic aortic occlusion. In the present experimental study, a Paratrend catheter was used to monitor CSF $P_O_2$ and $P_C_O_2$, and the results were correlated with ultrastructural changes occurring in the spinal cord after thoracic aortic clamping.

**Objective:** To study the correlation between intrathecal $P_O_2$ and ultrastructural changes in the spinal cord during thoracic aortic occlusion in pigs.

**Material and methods:** In 18 pigs, online intrathecal oxygenation was monitored by a multiparameter Paratrend catheter (Biomedical Sensors, High Wycombe, United Kingdom) during 60 minutes’ clamping of the proximal and distal descending thoracic aorta. The animals were randomly divided into 2 groups (A and B) depending on the level of distal aortic clamping. Distal aortic perfusion was restored through an aorto-iliac shunt, which also maintained low thoracic segmental perfusion of the spinal cord in group B. Perfusion-fixation technique was used before harvesting the spinal cord specimens, which later were evaluated with light and electron microscopy by an independent observer. Intrathecal parameters were interpreted as normal if $P_O_2$ was more than 0.8 kPa and $P_C_O_2$ was less than 12 kPa, as intermediate ischemia if $P_O_2$ was 0.8 or less or $P_C_O_2$ was more than 12 kPa, and as absolute ischemia if $P_O_2$ was 0.8 or less and $P_C_O_2$ was more than 12 kPa.

**Results:** Among 6 animals with ultrastructural changes of absolute spinal cord ischemia-reperfusion injury, 5 also had absolute ischemia according to variables derived by the Paratrend catheter. The 2 methods were in agreement in 3 of 5 animals with intermediate ischemia-reperfusion changes and in 5 of 6 animals with normal findings. The accuracy of cerebrospinal fluid $P_O_2$ and $P_C_O_2$ to predict electron microscopy–verified intermediate or absolute ischemia-reperfusion injury was 94%.

**Conclusions:** Monitoring of intrathecal $P_O_2$ after clamping of the descending aorta correlated with ultrastructural changes in the spinal cord in this pig model. (J Thorac Cardiovasc Surg 2001;121:316-23)
through an arterial-needle introducer a multiparameter PO2, bar arteries were also dissected. Abdominal visceral, lower intercostal (T-4 to T-13), and lumbar aorta together with the iliac arteries were dissected. The arch, and the entire descending thoracic aorta and abdominal aorta were clamped, and the solution (7 L) was delivered solely to the spinal cord specimens were then harvested from the cervical and lower thoracic spinal cord through laminectomies.

The animals were randomly divided into 2 groups depending on the level of the distal aortic clamp. The distal clamp was placed below L1 in group A (n = 9) and above T12 in group B (n = 9). After double aortic crossclamping, distal aortic perfusion was restored through the aorto-iliac shunt, which also maintained segmental perfusion of the spinal cord through segmental arteries (T12, T13, and L1) in group B. Double aortic crossclamping was maintained for 60 minutes followed by a 60-minute reperfusion period. Paratrend catheter recordings were continuously obtained during aortic crossclamping and reperfusion. No inotropic support, buffers, or vasodilators were given. At the end of the reperfusion period, 1000 mL of isotonic saline solution (37°C) was perfused followed by a fixative solution consisting of glutaraldehyde (2%) and paraformaldehyde (1%) in Millonig (300-mOsm buffer) solution with a pH of 7.4. These fluids were infused through the left subclavian artery with a pneumatic driven apparatus at a mean pressure of 70 to 80 mm Hg measured in the carotid artery. During the perfusion procedure, the distal aorta below L3 and all visceral arteries were clamped, and the solution (7 L) was delivered solely to the organs supplied by intercostal, subclavian, and carotid arteries. The blood and fixative solution were drained through the inferior vena cava. At the completion of perfusion, the pigs were killed with intravenous infusion of potassium chloride. The spinal cord specimens were then harvested from the cervical and lower thoracic spinal cord through laminectomies.

All animals were treated in compliance with the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The experiments also conformed with national guidelines. Approval of the study was obtained from the local committee of ethics for animal experiments.

Electron microscopy. The cervical and distal spinal cord segments were further incubated and transported for sectioning in the same fixative medium. The specimens were rinsed in a 300-mOsm Millonig solution (2.16 g NaH2PO4·2H2O, 18.51 g NaH2PO4·H2O, 6 g NaCl, and 1000 mL distilled water) before being sectioned in a Vibratome device (Plano, Oxford Laboratories, Foster City, Calif) to a thickness of 60 μm. After...
the specimens had been rinsed in the Millonig solution, postfixation for 20 minutes was performed twice in a 1% solution of osmium tetroxide in Millonig (300 mOsm) followed by ethanol dehydration in an ethanol series. The sections were infiltrated with Agar resin (Agar Scientific, Stanstead, United Kingdom) and embedded between acetate foils. Specimens were further sectioned to 2 µm in a Pyramitome 11800 device (LKB, Stockholm, Sweden) and stained with toluidine blue. Ultrathin sections to 30 to 40 nm were obtained in an Ultratom V 2088 device (LKB). The sections were then contrasted with uranyl acetate and lead citrate before being examined in a Hitachi H-7100 electron microscope (Hitachi Scientific Instruments, Nissei Sangyo Co, Ltd, Hitachi Ltd, Tokyo, Japan) at magnifications of 1000, 4000, 10,000 and 20,000.

Definitions of spinal cord ischemia. The specimens were initially evaluated with light microscopy especially to validate that they were sufficiently perfused with fixative solution. Ultrastructural examination of both ventral and dorsal horns was done with respect to the following changes: edema/vacuolization, axon degeneration, myelin separation, mitochondrial structural damage, and nuclear damage. All these changes were considered to be caused by ischemia-reperfusion and will henceforth be referred to as ischemic. A score of 0 (normal) or 1 (ischemic) was assigned to each of the aforementioned 5 different structural changes, thereby creating a possible minimum score of 0 and a maximum score of 5 if all variables were changed. Both ventral and dorsal horns were examined and summarized together in every animal. The observations from 1 control animal with a similar experimental model without spinal cord ischemia (eg, no aortic clamping) and the cervical spinal cord specimens that were not affected by ischemia-reperfusion were used as controls. The following scoring criteria for ultrastructural findings was established: 0-1 = normal; 2-3 = intermediate ischemia-reperfusion injury, and 4-5 = absolute ischemia-reperfusion injury. The CSF Po2 and PCO2 measurements obtained before declamping were used to define the aforementioned ischemic spinal cord changes. The summary of ultrastructural and Paratrend catheter–derived criteria for grading of spinal cord ischemia is given in Table I. A 2-way contingency table was used for comparison of parameters derived by the electron microscope and by the Paratrend catheter, as well as to calculate accuracy, sensitivity, and specificity, and negative and positive predictive values for Paratrend values in predicting ischemic spinal cord changes.

Results

Only 17 of the 18 randomized animals could be evaluated with the electron microscope because of a mechanical failure of the perfusion system in 1 animal in group B. The intention of this study model was to have 9 animals (group A) with absolute ischemia caused by exclusion of all collateral and segmental spinal cord blood supply with a distal clamp below L1. Absolute spinal cord ischemia with a CSF Po2 of 0.8 kPa or less and a PCO2 of more than 12 kPa was achieved in 4 of 9 animals in group A compared with 3 of 8 animals in group B (Tables II and III). Ultrastructural changes typical for normal, intermediate, and absolute ischemia-reperfusion injury are
shown in the micrographs of Figs 1 to 3. In animals with ultrastructural spinal cord changes, the pathologic changes were similar in ventral and dorsal horns.

The Paratrend values were stable during the last 30 minutes of the clamping period in all animals. The measurements obtained before declamping were used to define spinal cord ischemia. There was a good correlation between CSF Po2 and PCO2 measurements and electron microscopic evaluations as demonstrated in the 2-way contingency table (Table IV). Of 6 animals with ultrastructural changes of absolute spinal cord ischemia, 5 also had absolute ischemia according to variables derived
The 2 methods were in agreement in 3 of 5 animals with intermediate ischemia and in 5 of 6 animals with normal findings. No animals with normal CSF variables had intermediate or absolute ischemia-reperfusion changes according to ultrastructural findings. The accuracy, sensitivity, and specificity of Paratrend catheter–derived values in predicting electron microscopy–verified changes of intermediate or absolute spinal cord ischemia-reperfusion were 94%, 100%, and 83%, respectively. The negative and positive predictive values were 100% and 92%, respectively (Table IV).

Table II. CSF PO$_2$ and PCO$_2$ values and ischemia-reperfusion changes in the spinal cord according to Paratrend (PT) catheter and electron microscopic (EM) evaluation (group A)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline PT PO$_2$ (kPa)</th>
<th>DAXC 60 min</th>
<th>Baseline PT PCO$_2$ (kPa)</th>
<th>DAXC 60 min</th>
<th>Classification according to:</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2.6</td>
<td>0</td>
<td>6.67</td>
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<tr>
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<td>27.00</td>
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<tr>
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<td>11.80</td>
<td>I</td>
</tr>
<tr>
<td>7</td>
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<tr>
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<tr>
<td>9</td>
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<td>0.8</td>
<td>6.2</td>
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</table>

Table III. CSF PO$_2$ and PCO$_2$ values and ischemia-reperfusion changes in the spinal cord according to Paratrend (PT) catheter and electron microscopic (EM) evaluation (group B)

<table>
<thead>
<tr>
<th>Animal</th>
<th>Baseline PT PO$_2$ (kPa)</th>
<th>DAXC 60 min</th>
<th>Baseline PT PCO$_2$ (kPa)</th>
<th>DAXC 60 min</th>
<th>Classification according to:</th>
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<tr>
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Table IV. Two-way contingency table

<table>
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<tr>
<th>Histopathology</th>
<th>Normal</th>
<th>Ischemic</th>
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</thead>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>Normal</td>
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<td>1</td>
</tr>
<tr>
<td>Ischemia-reperfusion</td>
<td>0</td>
<td>11</td>
</tr>
</tbody>
</table>

Correlation between Paratrend catheter and electron microscopic findings.

Discussion

Predicting the degree of spinal cord ischemia during thoracic aortic clamping is of importance to obtain better neurologic outcome. Various neurophysiologic monitoring methods have been successfully used during aortic replacement but are still controversial and not universally accepted. We have recently described the use of a flexible multiparameter intrathecal catheter for continuous monitoring of cerebrospinal PO$_2$, PCO$_2$, and pH. This method was validated with epidural laser Doppler flowmetry. The percutaneous use of the Paratrend catheter was also reported in a patient undergoing thoracoabdominal aortic surgery. Recently, Ishizaki and coworkers have reported the use of a rigid mass spectrometer probe for CSF PO$_2$ measurements during experimental aortic occlusion for identification of
spinal cord feeding arteries. Perfusion of these arteries improved CSF PO$_2$ and spinal cord evoked potentials.

The present study was designed to evaluate the correlation between CSF parameters derived with the Paratrend catheter and structural changes in the spinal cord in a previously reported aortic occlusion model in pigs.$^1$ Other studies on spinal cord ischemia have demonstrated a satisfactory correlation between histopathologic spinal cord changes and functional outcome evaluated by use of the Tarlov criteria.$^{17-22}$ Yamamoto and coworkers$^{21}$ observed no morphologic ischemic spinal cord degeneration if normal hind leg function was present. On the other hand, in animals with flaccid paraplegia, axonal loss, myelin breakdown, and vacuolization were prominent. Only light microscopy was used in that study. Routine paraffin sections might be acceptable for evaluation of relatively severe spinal cord damage but are inadequate for the examination of nerve fiber morphology. This requires the use of resin-embedded sections with osmium tetroxide postfixation and/or electron microscopy.$^{22}$

Transmission electron microscopy was used in this study to investigate the correlation between CSF oxygenation and ischemic spinal cord changes. Naslund and associates$^{23}$ demonstrated that the ventral horn from neurologically intact animals had a normal microscopic appearance compared with that of animals with paraplegia after spinal cord ischemia, in which destruction of ventral horn cells and pronounced vacuolization were apparent. Intermediate ischemia of the spinal cord was observed in that study in some of the animals with delayed-onset paraplegia. Balentine$^{24}$ described axon and myelin affection after ischemic spinal cord injury but admitted that irregular splitting sometimes was seen even in controls, which were separable from artifacts. This observation clearly reveals some of the difficulties of interpreting the histopathologic or ultrastructural changes aimed to define reversible and irreversible changes. To minimize the postmortem artifacts that can arise during the removal of specimens, we used a special perfusion-fixation technique in the present study. Turren$^{25}$ as early as 1936 showed that if spinal cord ischemia was present for more than 20 minutes in cats, there was no neurologic recovery after 24 hours and histologic changes were severe. He concluded that reperfusion injury influenced morphologic appearance; therefore, the time between the ischemic event and harvesting of the specimen might influence the ultrastructural picture. Cellular swelling but not interstitial edema was correlated to the presence of neurologic dysfunction.

Because of some of the aforementioned minor histopathologic changes in neurologically intact animals in previous studies, our observation from the control animals, and the evaluation of normal cervical spinal cord segments, we decided to classify electron microscopic appearance as normal in those with 0 or 1 scoring unit. Scoring unit 1 was usually attributed to minor vacuolization without any other changes. We did not observe severe vacuolization of axons without any other ultrastructural changes, as mentioned in the study by Balentine$^{24}$ in some of the neurologically intact animals. In the present study, severe vacuolization of the axons was always accompanied by other signs of severe ischemia-reperfusion injury. The different types of ultrastructural changes observed in the ischemic group with a score of 4 to 5 were in agreement with the previous studies. These changes consisted mainly of increased density of neurons, vacuolization of neuronal perikarya, axonal dark degeneration, and severe myelin sheet separation. The mitochondria were usually enlarged or had deranged internal structure. The absolute ischemia group had severely deranged ultrastructure, and the changes seemed likely to be irreversible. Different types of cell deaths have been described to occur depending on the cause of insult: hypoperfusion, reperfusion, or both.$^{26}$ After spinal cord ischemia due to hypoperfusion, some neuronal cells may recover metabolic activity but then later die of apoptosis during the reperfusion period. This issue cannot be addressed in the present study, because all the specimens were harvested after a 60-minute reperfusion period.

The results of the present study indicated that intrathecal PO$_2$ monitoring during aortic clamping in this experimental model correlated with spinal cord changes evaluated by electron microscopy. It is interesting that the negative predictive value of CSF PO$_2$ and P$_{CO_2}$ measurements during aortic occlusion evaluated against ultrastructural changes was 100%. No animals with normal CSF PO$_2$ and P$_{CO_2}$ measurements also had severe morphologic changes. In animals with intermediate ischemia according to Paratrend values, different ultrastructural pictures were seen, varying from normal to intermediate ischemia-reperfusion changes. It is impossible to speculate on the possible clinical relevance of intermediate ultrastructural changes.

Reliable data on the critical level of CSF PO$_2$ and P$_{CO_2}$ is not available in the literature. Interstitial PO$_2$ less than 0.8 kPa has been discussed by Valadka and associates$^{27}$ and, in an editorial view, has been suggested to indicate a high risk of ischemic brain injury.$^{28}$ In
a recent review by Tønnessen, the maximum aerobic tissue PcO₂ was proposed to be 10 to 12 kPa with a range of 9 to 19 kPa. The suggestions from these previous studies were the reason for our selecting arbitrary values for grading spinal cord ischemia in the present experimental model, and these values correlated well with electron microscopic findings. These observations are in agreement with our findings observed in a previous study. Future studies with functional outcome using Tarlov criteria are needed for further evaluation of the predictive power of Paratrend catheter–derived parameters with respect to neurologic outcome after spinal cord ischemia. Future studies with functional outcomes are also needed for better definition of cutoff values.

In conclusion, CSF PO₂ monitoring during thoracic aortic clamping correlated with ultrastructural changes in this pig model. The difference between the present method of CSF PO₂ monitoring and the previous ones is the type of intrathecal catheter. The flexible multisensor Paratrend catheter used in this study has a small diameter (0.5 mm) and can easily be applied with a percutaneous technique in human beings.

We gratefully acknowledge the technical assistance of laboratory technician Monica Hall throughout the experiments. We also appreciate the invaluable contribution of Kärstin Flink and Kerstin Rystedt for their careful and time-consuming preparation of spinal cord specimens.

REFERENCES

23. Naslund TC, Hollier LH, Money SR, Facundus EC, Skenderis
27. Valadka AB, Gopinath SP, Contant CF, Uzura M, Robertson CS.