Induction of heat shock protein 70 in the rat brain following intracisternal infusion of autologous blood: evaluation of acute neuronal damage

PETRA M. KLINGE, M.D., HEIKE BECK, M.D., THOMAS BRINKER, M.D., PH.D., GERHARD F. WALTER, M.D., PH.D., AND MADJID SAMII, M.D., PH.D.

Department of Neurosurgery, Nordstadt Hospital, Hannover, Germany, and Departments of Neurosurgery and Neuropathology, Hannover Medical School, Hannover, Germany

Object. Investigation into a potential treatment for the acute period following onset of spontaneous subarachnoid hemorrhage (SAH) is hampered by the lack of a standardized experimental model. For that purpose the authors elaborated on a small-animal model in which computer-controlled intracisternal blood infusion is used and investigated whether this model can reliably reproduce acute neuronal injury after SAH.

Methods. Whole autologous blood (blood-infused group) or isotonic saline (control group) was infused into the cisterna magna or olfactory cistern of rats. The infusions decreased exponentially during a 5-minute period. Throughout the infusion period, intracranial pressure (ICP) was monitored. Neuronal injury was quantified by observing tissue immunoreactivity to a 70-kD heat shock protein (HSP70) and comparing this with the tissue’s reaction to hematoxylin and eosin staining. On Days 1, 3, and 5, the CA1, CA3, and dentate gyrus regions of the hippocampus were analyzed, respectively.

During saline infusion ICP increased within seconds beyond 80 mm Hg and afterward decreased in accordance with the infusion rate. During the infusion of blood, the same initial pressure peak was found, but the ICP remained increased beyond this pressure level throughout the 5-minute infusion period. The HSP70 immunoreactivity in the saline-infused group was found only on Day 1 in the CA1 region and the dentate gyrus, but not in the CA3. After injection of whole blood, there was HSP70-positive staining in the CA1, CA3, and dentate gyrus regions throughout the observation period.

Conclusions. The controlled cisternal infusion of blood caused neuronal injury that resembled that of previous experimental models that produce SAH by rupture of intracranial vessels with endovascular techniques. Unlike those experiments, the intracisternal infusion technique presented by the authors provides more standardized bleeding with regard to ICP, the volume of subarachnoid blood, and the extent of acute cellular injury.

Key Words • subarachnoid hemorrhage • heat shock protein • cerebral ischemia • rat

The mechanisms of neuronal injury from delayed ischemia, such as those caused by vasospasm, that occur in spontaneous subarachnoid hemorrhage (SAH) have been extensively investigated. Nevertheless, little is known about the pathophysiological mechanisms occurring during the acute period following aneurysm rupture. This is important because the patient’s prognosis is predominantly affected by the initial phase after SAH. In fact, some authors have suggested that a short-lasting nonphysiological peak in intracranial pressure (ICP) after aneurysm rupture causes severe global ischemia. However, others have postulated that primary ischemic damage results from acute vasospasm.

Unfortunately, we still lack a standardized experimental model that simulates the acute situation and the related pathophysiological sequelae after aneurysm rupture.

Recently, a model of SAH in rats was introduced in which bleeding was produced by endovascular perforation of intracranial vessels. To detect early neuronal damage, the authors used the immunoreactivity of a heat shock protein with a molecular weight of 70 kD (HSP70), which is known to be a sensitive marker of neuronal injury. There is some indication that these experiments reliably mimic the severe acute ischemia that is clinically expected to follow aneurysm rupture because a pattern of cellular injury was found that resembled findings in rats after temporary bilateral carotid ligation. A major disadvantage of endovascular techniques, however, lies in the fact that significant variability has been observed between animals with respect to the extent of acute cellular injury; ICP increase, and cerebral blood flow (CBF). Thus, the variable pattern of injury limits the opportunity to perform the quantitative investigations that are required for pharmacological studies.

As an alternative model of SAH, the injection of autologous blood into the cisterns provides more standardized conditions with regard to the volume of subarachnoid blood and acute ICP increase. When given as a bolus into the cisterna magna, however, only lysed blood, and not whole blood, has been observed to produce cellular injury. An additional disadvantage of this technique is that bolus injections only cause a short considerable increase in ICP; this does not resemble the ICP plateau that lasts several minutes after aneurysm rupture in patients.
To serve clinical purposes, we elaborated on a new animal model that produces SAH by using a longer-lasting intracisternal infusion of autologous blood. The rate of infusion was adjusted according to previous findings that had demonstrated that the bleeding rate after aneurysm rupture decreases within minutes toward zero in an exponential fashion.\(^1\),\(^2\) Preliminary experiments in cats have already demonstrated that by using such an infusion technique, an immediate ICP increase with a subsequent minutes-long plateau at the level of the arterial blood pressure can be obtained.\(^3\)

We investigated acute neuronal injury by using HSP70 immunostaining in combination with conventional histological methods. The specific question posed was whether it might be possible to reproduce acute neuronal injury by using this controlled intracisternal infusion technique, as found in experiments in which endovascular rupture of intracranial vessels is used.

Materials and Methods

**Experimental Techniques**

A total of 124 adult Wistar rats, each weighing 270 to 330 g, were used in this study. All procedures were approved by our accredited animal care committee. Anesthesia was induced in the animals by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (8 mg/kg) and was maintained by supplemental doses of ketamine (25 mg/kg). Throughout the experiments the rat’s heart rate and mean arterial blood pressure were monitored continuously. Additionally, body temperature was measured and maintained at 37 ± 0.5°C by placing the animals on a heating pad. A permanent nylon catheter with an inner diameter of 0.58 mm and an outer diameter of 1.02 mm was inserted stereotactically into each rat’s cisterna magna or olfactory cistern at a depth of 6 or 10 mm, respectively. To aid the insertion, the animals’ skin was shaved and incised medially to place a burr hole, 2 mm in diameter, into the interparietal and frontal bone, respectively. The catheter was sealed in a watertight fashion with cyanoacrylate adhesive.

Using a three-way stopcock, the catheters were connected to a pressure transducer for ICP monitoring. To compensate for leakage of cerebrospinal fluid (CSF) caused by catheter insertion, repeated bolus injections of 0.02 ml of isotonic saline were given until a stable baseline ICP was reached. Thus, experimental conditions were usually achieved 30 minutes after initial catheter placement.

Infusion of 0.75 ml of isotonic saline or autologous whole blood that had been obtained from each rat’s tail artery was accomplished during a 5-minute period, during which the rate of infusion decreased exponentially, as shown in Fig. 1. Controlled infusion rates were obtained using an IBM-compatible personal computer, an adjustable pump, and commercially available laboratory software.

Monitoring of ICP and mean arterial blood pressure was continued until baseline levels of both parameters were reached and maintained for at least 10 minutes. The permanent ventricular catheters were left in place.

**Histological and Immunocytochemical Analysis**

To detect neuronal injury by eosinophilic degeneration and nuclear pyknosis in the hippocampal regions, hematoxylin and eosin were used to stain tissue samples obtained in six animals after injection of saline and in 10 animals after injection of whole autologous blood into the cisterna magna.

Briefly, the rat brains were removed via a cardiac catheter after perfusion with 4% phosphate-buffered formaldehyde and maintained in the same solution at 4°C for 4 days. Paraffin-embedded 5-µm-thick sections were cut, dehydrated, delipidized, rehydrated, and counterstained with hematoxylin and eosin. Sections were then dehydrated and placed on coverslips with Entellan medium.

Morphologically damaged and intact neurons in the hippocampal pyramidal layer were counted using × 40 microscopic magnification. Neuronal cell injury was expressed as the relative amount of damaged cells in each anatomically defined hippocampal region (CA1, CA3, and dentate gyrus) in percentages as follows: 0 to 5% (none), 6 to 20% (mild), 21 to 50% (moderate), and greater than 50% (severe).

For the immunohistological analysis, we examined 108 male Wistar rats, half of which had the catheter placed in the cisterna magna and the other half of which had the catheter inserted into the olfactory cistern. These rats were subdivided into three groups of 18 animals each: sham-operated (catheter insertion only), saline infused, and blood injected. In each group, six animals were examined on Day 1, six on Day 3, and six on Day 5.

The HSP70 immunostaining was performed according to the method described by Matz, et al.,\(^4\) with slight modifications. The rats were anesthetized (see earlier description) and perfused via a cardiac catheter filled with isotonic saline, heparin (0.5 ml), and 2% Scandanica (0.2 ml) followed by 4% phosphate-buffered parformaldehyde (400 ml). The brains were postfixed in 30% saccharose solution at 4°C for 48 to 72 hours. The brains were removed and cryofixed in Isopentan-chilled nitrous at −70°C and cut into 5-µm sections. The sections were dried overnight at room temperature and then covered with aluminum film and maintained at −20°C.

Sections were placed in phosphate buffer (PB) containing 2% sheep serum (SS)/0.1% bovine serum albumin, and 0.2% Triton-X used as “blocking agents” to unspecific protein reactions, for 2 hours at room temperature. This was followed by a 48-hour incubation at 4°C with HSP70 monoclonal antibody diluted to a 1:1000 concentration in PB-SS. After three 5-minute washes in PB, the sections were incubated with biotinylated sheep anti–mouse immunoglobulin G antibody at a concentration of 1:200 in PB-SS for 2 hours. All sections were washed again three times for 5 minutes each with PB, placed in avidin-horseradish-peroxidase solution for 3 hours, washed in PB three times for 5 minutes each, and reacted for horseradish peroxidase with 0.015% diaminobenzidine solution and 0.001% hydrogen peroxide for 3 minutes. The sections were dehydrated and placed on coverslips with Entellan. Negative controls were provided by omitting the primary antibody. Counterstaining was not performed to facilitate subsequent image analysis.

Image analysis was performed according to methods previously published by others.\(^5\),\(^6\)

Histological sections were viewed with the aid of a photomicroscope using × 40 objective and digitally captured. A public-domain

---

P. M. Klinge, et al.

*J. Neurosurg. / Volume 91 / November, 1999*
image-analysis program (Scion Image) was used to quantify HSP70 immunostaining.

The most relevant steps of image analysis included: 1) conversion into a gray scale with gray levels ranging from 0 to 255 by image segregation with automatic true-color analysis; 2) thresholding via optical density at a level that distinguished between stained and unstained parts; and 3) binary image processing. The total HSP70-positive areas were expressed as relative values resulting from the quotient of the stained area and defined reference area.

Statistical Analysis

To evaluate the effects of the experimental groups (sham-operated, saline-infused, and blood-infused), anatomical region (CA1, CA3, and dentate gyrus), and time (1, 3, and 5 days) on HSP70 immunostaining we used analysis of variance (F statistic for multiple-group comparison). Post hoc pair-wise comparisons were made using Fisher’s protected least-significant difference test (multiple t statistic).

For analysis of ICP changes as a function of time, we used analysis of variance with repeated measures.

Sources of Supplies and Equipment

We purchased permanent nylon catheters from Portex (Hythe, UK) and used cyanacrylate adhesive obtained from Henkel (Düsseldorf, Germany) to seal them. The adjustable pump and DasyLab software used to control infusion rates were obtained from Watson and Marlow (Falmouth, UK) and Datalog (Mönchengladbach, Germany), respectively. Sigma (Deisenhofen, Germany) manufactured the Triton-X and phosphate-buffered formaldehyde and Merck (Darmstadt, Germany) the Entellan; Amersham (Braunschweig, Germany) produced the HSP70 monoclonal antibody and the biotinylated sheep anti–mouse immunoglobulin G antibody. The avidin–horseradish peroxidase solution was obtained from ICN (Eschwege, Germany). Histological sections were examined using a microscope obtained from Zeiss (Oberkochen, Germany). Image analysis was performed using Scion Image software for Windows (version 1.58) available from the National Institutes of Health, Bethesda, Maryland.

Results

Course of ICP During Intracisternal Infusion of Saline and Blood

Baseline ICPs after catheter insertion were 7.6 ± 2.6 mm Hg in the cisterna magna and 7.3 ± 2.1 mm Hg in the olfactory cistern group. After injection of blood into the cisterna magna, ICP increased up to levels of 82.9 ± 13.4 mm Hg in the saline-infused group and 72.3 ± 16.3 mm Hg in the blood-infused group within seconds after infusion began. The ICP increase and ICP peak after blood infusion into the olfactory cistern were slightly lower than those in the cisterna magna. Having reached their initial peaks, the ICP courses showed statistically significant differences between the blood- and saline-infused groups in both catheter placements (p < 0.0001 in the cisterna magna group and p < 0.001 in the olfactory cistern group, respectively). In the saline-infused group, ICP continued to increase and reached its maximum level of 140 mm Hg in the cisterna magna and 130 mm Hg in the olfactory cistern after 2.5 minutes, followed by a slow decrease to approximate levels of 80 mm Hg and 40 mm Hg after 5 and 10 minutes, respectively (Fig. 2).

Regarding catheter placement in the cisterna magna and the olfactory cistern, only negligible differences were found.

Staining With Hematoxylin and Eosin

The results of the blood-infused group 5 days after infusion are shown in Fig. 3. A severe neuronal eosinophilic degeneration and cell damage with nuclear pyknosis necrosis were observed exclusively in the CA1 region in 10% of animals. Moderate neuronal injury was found in the CA1 and dentate gyrus regions in 20% and 10% of animals, respectively. A mild injury was found in all regions, including the CA3, in 30% of animals. In most animals, only a slight neuronal injury (< 5%) was observed predominantly in the CA3 region.

In the saline-infused group, no neuronal damage was observed. The typical cellular pattern of hematoxylin and eosin staining is shown in Fig. 4.
Heat Shock Protein 70 Immunoreactivity

Because there was no baseline expression of HSP70, positive neurons were easily identified, as shown in Fig. 5. The HSP70-positive neurons were localized in the pyramidal cells of the hippocampus (CA1, CA3, and dentate gyrus).

Differences in regional immunoreactivity among the sham-operated, saline-infused, and blood-infused groups were most striking with respect to the CA1 and the CA3 regions. The specific time course of HSP70 immunoreactivity was more obvious in the dentate gyrus than in the CA1 and the CA3 regions.

The values of immunoreactivity resulting from morphometrical analysis are summarized in Table 1. Significant findings are shown in Figs. 6 and 7 and will be discussed in the next section.

Comparison of Immunoreactivity in the CA1 and CA3 Regions

In the cisterna magna group, sham-operated animals showed a slight immunostaining in both the CA1 and CA3 regions.

After infusion of saline, immunostaining was more pronounced in the CA1 than in the CA3 region (p = 0.05). In comparison with the sham-operated group, immunoreactivity in the CA1 region was statistically significantly increased (p < 0.03).

After injection of blood, HSP70 immunostaining was increased in the CA1 and CA3 regions at nearly equal levels. Although immunoreactivity in the CA1 region was only statistically different from the sham-operated group (p < 0.001), the immunoreactivity in the CA3 region was statistically significantly different from both the sham-
Cellular injury in experimental SAH

TABLE 1

<table>
<thead>
<tr>
<th>Time Course of Immunoreactivity in the Dentate Gyrus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-Operated</td>
</tr>
<tr>
<td>---------------</td>
</tr>
<tr>
<td>Day 1</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA3</td>
</tr>
<tr>
<td>dentate gyrus</td>
</tr>
<tr>
<td>Day 3</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA3</td>
</tr>
<tr>
<td>dentate gyrus</td>
</tr>
<tr>
<td>Day 5</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA3</td>
</tr>
<tr>
<td>dentate gyrus</td>
</tr>
</tbody>
</table>

*Explanation of relative values is shown in Materials and Methods.

Operated (p < 0.001) and saline-infused (p < 0.01) groups (Fig. 6 upper).

After catheter placement into the olfactory cistern, findings were minimally different: in the sham-operated group, HSP70 immunoreactivity was slightly more pronounced in the CA1 region than in the CA3; however, regional differences within the experimental groups did not reach statistical significance. Statistically significant group differences were only found between the blood-infused and sham-operated groups as well as between the CA1 (p = 0.05) and CA3 (p < 0.05) regions, respectively (Fig. 6 lower).

Time Course of Immunoreactivity in the Dentate Gyrus

In animals in which the catheter had been placed in the cisterna magna, immunoreactivity in the sham-operated group remained at a low baseline level with a slight increase on Day 5.

Although in the saline-infused and blood-infused groups, levels of HSP70 immunostaining varied among Days 1, 3, and 5, the effect of timing was not significant.

Statistically significant differences were only observed between groups at the different time points (p < 0.0001; Fig. 7 upper).

Already on Day 1, there was an initial increase in HSP70 immunostaining after injection of saline. The initial increase reached statistical significance compared with the sham-operated group (p < 0.01). By Day 3 immunoreactivity had decreased to the level of the sham-operated group, with no statistical difference (Fig. 7 upper).

After infusion of blood, the differences in HSP70 immunoreactivity compared with the saline-infused and sham-operated groups increased from Day 1 to Day 5. The staining levels of the blood-infused group were always statistically increased compared with those of the saline-infused group (p < 0.05 on Day 1; p < 0.001 on Day 3; and p < 0.01 on Day 5) and the sham-operated group (p < 0.0001 on Days 1 and 3; and p < 0.01 on Day 5) (Fig. 7 upper).

In animals in which an olfactory cistern catheter had been placed, differences concerning relationships between groups and specific time courses were less pronounced with minimal qualitative differences, compared with animals in which a cisterna magna catheter had been placed (Fig. 7 lower).

On Days 1 and 3, there only were significant differences between the blood-infused and sham-operated groups (p < 0.05).
Furthermore, sham-operated animals with olfactory cistern catheters demonstrated an overall increased immunoreactivity in comparison with sham-operated animals with catheters in the cisterna magna. This difference, however, did not reach statistical significance.

On Day 3, there was a slight decrease in immunoreactivity after blood infusion in animals with an olfactory cistern catheter, whereas there was a slight increase in immunoreactivity in those with a catheter in the cisterna magna.

Discussion

In the present study, we investigated whether controlled cisternal infusion of whole blood causes an identical neuronal injury to that found after scratching intracranial vessels by endovascular techniques.16

Using HSP70 immunoreactivity, we actually found a temporal and spatial pattern of HSP70 staining that was quite similar to that of endovascular SAH models.

The observed neuronal injury pattern, furthermore, resembled findings after global cerebral ischemia from temporary vessel ligation: in the early period, the CA1 and dentate gyrus regions were specifically affected, whereas the CA3 region was more resistant to the initial impact of SAH.

Fig. 7. Bar graphs demonstrating the time course of HSP70 immunoreactivity (mean ± SD) in the dentate gyrus on Days 1, 3, and 5 in the sham-operated, saline-infused, and blood-infused groups after catheter implantation into the cisterna magna (upper) and the olfactory cistern (lower). For statistical differences see text.

Furthermore, sham-operated animals with olfactory cistern catheters demonstrated an overall increased immunoreactivity in comparison with sham-operated animals with catheters in the cisterna magna. This difference, however, did not reach statistical significance.

On Day 3, there was a slight decrease in immunoreactivity after blood infusion in animals with an olfactory cistern catheter, whereas there was a slight increase in immunoreactivity in those with a catheter in the cisterna magna.

Infusion Rate and ICP Increase

Following aneurysm rupture, the abrupt and minutes-long increase in ICP up to the level of arterial blood pressure has been shown clinically10,25 and experimentally1,2,23,24 Such an ICP pattern cannot be simulated by bolus injection techniques or by the constant-rate infusion of a reasonable volume of blood (1–3 ml/kg body weight) into the cisterna magna. Bolus injections of such volumes cause only a short, seconds-long peak, far beyond the level of the arterial blood pressure; constant-rate infusions provide a continuous ICP increase, but do not mimic the sudden rise in ICP or the subsequent minutes-long pressure plateau with consecutive global cerebral ischemia.3,4

The present infusion technique was developed on the basis of experimental observations on bleeding after aneurysm rupture. It was found that the bleeding rate starts at a high level and decreases exponentially within several minutes.12,23 This experimental SAH technique already has been successfully performed in cats.2 To obtain a small-animal model, we adapted the infusion curve to the different CSF dynamics of the rat until we were able to provide a 5-minute ICP plateau at a pressure level of at least 80 mm Hg.

We only found this ICP pattern only during infusion of whole blood and not during infusion of saline. This suits the expected changes in CSF dynamics caused by subarachnoid blood clots resulting in acute impairment of CSF absorption and exhaustion of the intracranial volume buffering capacity.5

The observed ICP course after controlled blood infusion did not differ with respect to whether the catheter was placed in the cisterna magna or the olfactory cistern. However, HSP70 immunoreactivity in sham-operated animals with an olfactory cistern catheter was increased compared with that found in animals in which the catheter had been inserted into the cisterna magna, indicating some minor trauma caused by catheter insertion into the olfactory cistern.

Heat Shock Protein 70 Immunoreactivity

Presently, very little is definitely known about the functional significance of HSPs, but they are commonly supposed to be involved in cellular protection because cells with expression of HSPs have survived after significant ischemia, whereas cells without HSPs have not.7,12,15

A certain hierarchy of cell types and anatomical sites of ischemic stress was also observed using HSP staining methods, a phenomenon that has been explained according to the concept of “selective vulnerability.”9,11 Neurons have been shown to produce HSPs within the first minutes after global and regional ischemia, whereas glial cells remain negative even after 10 minutes.7 Thus, neuronal HSP70 immunoreactivity precedes morphological damage detected via conventional staining methods.15

All these findings indicate that HSPs are sensitive to acute neuronal injury and have the potential to indicate recovery from different types of physiological stress (hypoglycemia) and pathological stress (ischemia, narcotic agents, mechanical trauma).20 As such, HSPs were introduced in experiments that specifically focused on acute cellular reactions, such as neuronal damage in the acute period after spontaneous SAH.16,11

P. M. Klinge, et al.

J. Neurosurg. / Volume 91 / November, 1999
Cellular injury in experimental SAH

Using HSP70 staining in our study, we were able to confirm these observations. Significant differences in regional distribution and in the initial and prolonged time course of cellular injury were found following cisternal infusion of whole autologous blood and saline.

Because the animal’s blood glucose level and the narcotic agent ketamine have an effect on HSP70 induction, staining results in our animals might have been influenced at least by these parameters. All experimental groups were subjected to the same surgical procedure and anesthetic protocol; therefore, group differences in HSP70 immunoreactivity could only be a result of the different infusion agents (none, saline, or blood).

Regional Distribution and Time Course of HSP70 Immunoreactivity

In the saline-infused group, the CA1 region and not the CA3 region showed significant immunofluorescence labeling as early as Day 1. This contributes to findings in sublethal ischemia, in which the CA1 region, the thalamus, and also the caudate putamen have been the most vulnerable areas.

The selective anatomical hierarchy of HSP70 immunostaining vanished after infusion of blood. Significant immunoreactivity was found in both the CA1 and CA3 regions. This phenomenon was also observed after experimental bilateral carotid ligation, indicating severe global ischemia.2,3

Regarding the difference in ICPS increases during infusion of saline and blood, we have assumed that the observed regional HSP70 pattern resulted from an ICP-dependent CBF decrease. However, there are contrary observations emphasizing that early CBF impairment after SAH and the resulting neuronal damage are due to acute vasospasm and are related to ICP.1

Specifically, the role of acute vasospasm as a cause of initial CBF perturbation in spontaneous SAH was postulated because reduced CBF was found even under conditions of normal ICP.25 Nevertheless, a major limitation of these experimental settings lies in the fact that the researchers could not find or did not demonstrate any significant neuronal injury. The lack of neuronal damage after bolus injection of whole blood was clearly documented.11

This finding of negative HSP70 staining indicates indirectly that a significant CBF impairment, which actually causes neuronal injury, could only be expected in the vicinity of prolonged severe ICPS increases, which cannot be achieved by bolus injections.4

Measurements of CBF in different SAH models have shown that, in the presence of a longer-lasting pressure plateau, severe impairment of CBF can actually be achieved if ICPS exceed 80 to 100 mm Hg during SAH.1,3

We therefore assume that there was an ICPS-dependent ischemic period lasting at least 5 minutes in the blood-infected animals, even though we did not perform CBF measurements in our experiments.

Concerning the pathophysiological effects of acute ischemia following SAH, we therefore question the significance of acute vasospasm as a cause of temporary or permanent neuronal injury in spontaneous SAH as cited by other investigators.21

Conclusions

With the presented intracisternal infusion technique we were able to maintain a prolonged ICP increase up to levels of arterial blood pressure, thus simulating the ICP found after aneurysm rupture.

Because the regional HSP70 staining pattern in our experimental model closely resembled findings after temporary bilateral carotid ligation, we assume that ischemia was the main stress factor in neuronal injury in our animals. Thus, with intracisternal infusion, we were able to simulate the initial phase of SAH in the same manner obtained from endovascular SAH techniques.

A major advantage of the cisternal infusion technique is that it provides greater reproducibility with respect to ICP increase, the amount of released blood, and the extent of cellular injury.

References


J. Neurosurg. / Volume 91 / November, 1999


Manuscript received February 2, 1999. Accepted in final form July 12, 1999. This study was supported by the Deutsche Forschungsgemeinschaft (BR1416 1-2).

Address reprint requests to: Thomas Brinker, M.D., Department of Neurosurgery, Nordstadt Hospital Hannover, Haltenhoffstrasse 41, 30167 Hannover, Germany.