An Improved Canine Model of Subarachnoid Hemorrhage Using Intrathecal Indwelling Catheters

Takashi MORI, Takao ASANO[1)], Kazuya NAGATA[2)], Takuo ISHIDA[3)], and Tohru ABE

Departments of Internal Medicine and [1)]Neurosurgery, Saitama Medical Center/School, 1981 Kamoda, Kawagoe-city, Saitama 350, [2)]Department of Neurosurgery, Showa General Hospital, 2–450 Tenjin-cho, Kodaira-city, Tokyo 187, and [3)]Department of Clinical Pathology, Nippon Animal Science University, 1–7–1 Kyonan-cho, Musashino-city, Tokyo 181, Japan

(Received 6 February 1997/Accepted 13 May 1997)

ABSTRACT. In the present study, the feasibility of intrathecal indwelling catheters in the preparation of a repeated subarachnoid hemorrhage (SAH) model in dogs, as well as chronic intrathecal administration of therapeutic agents against the ensuing cerebral vasospasm was examined. Briefly, through a small suboccipital incision, two catheters were introduced into the subarachnoid space so that their tips were positioned in the prepontine cistern. One was used to induce SAH by infusing autologous blood, and the other to administer pharmacological agents (saline and/or saline containing a dye in this study) by means of an osmotic pump. The occurrence of cerebral vasospasm was followed by angiography via the catheter placed in the vertebral artery. The obtained results show: i) the injected blood effectively formed a subarachnoid clot in the prepontine cistern, invariably leading to the occurrence of severe cerebral vasospasm of the basilar artery; ii) the fluid injected by the osmotic pump was evenly distributed in the cisterns around the brain stem; iii) on post mortem pathological examination, no injury of the brain or the major arteries ascribable to the placement of catheters was found. Therefore, the present model is considered to be useful for both the investigation of pathophysiology and therapy of cerebral vasospasm following SAH, to be more favorable from the standpoint of animal protection, and more convenient and reliable than those used until now. — KEY WORDS: canine, cerebral vasospasm, osmotic pump.

J. Vet. Med. Sci. 59(9): 825–828, 1997

Cerebral vasospasm is one of the most serious complications of aneurysmal subarachnoid hemorrhage (SAH) [13, 14]. While the underlying pathogenetic mechanism as well as the efficacy of pharmacological treatment have been investigated using various SAH models in animals, the most frequently used has been the canine "two-hemorrhage" model, in which SAH is induced on two separate occasions by successive cisternal injection of autologous blood several days apart [9–12, 18]. This technique, however, has the drawbacks that it requires repeated anesthesia and operative interventions, and that the second cisternal puncture tends to be difficult because of the presence of a clot and, frequently aseptic meningitis within the subarachnoid space. In this regard, it occurred to us that the use of intrathecal indwelling catheters might make the induction of SAH as well as the intrathecal delivery of pharmaceutical agents easier and surer. Thus, the present study was undertaken to examine whether this technique would serve the purpose of inducing reproducible SAH with ensuing cerebral vasospasm, and delivering the test agent uniformly into the subarachnoid cisterns around the brain stem.

Sixteen adult beagle dogs of either sex, weighing 10 to 15 kg, were assigned to three groups: a C-SAH group, a SAH model using the conventional technique (6 dogs); a P-SAH group, a SAH model using the present technique (6 dogs); a sham group, receiving only a sham operation (4 dogs). The environment was controlled, with a temperature of 22 ± 2°C, a relative humidity of 55±10%, and 10 times/hr a fresh air change with a 12-hr fluorescent light/dark cycle. All animals were fed a commercial dry-type dog diet (Ajinomoto General Foods, Inc., Tokyo, Japan) and tap water.

The care of the animals and procedures in the study complied with the "Principle of Laboratory Animal Care" and the "Guide for the Care and the Use of Laboratory Animals" issued by the National Institute of Health (U.S.A. DHHS publication No. [NIH] 85–23, revised 1985).

Under general anesthesia (sodium pentobarbital 30 mg/kg, i.v.) and mechanical ventilation, the head of each dog was placed in a supine position using a stereotaxic device (Takahashi Co., Ltd., Tokyo, Japan). Arterial blood gas levels were monitored to safeguard against pO_2 fluctuation in the dimentions of the cerebral artery due to variations in pCO_2. Rectal temperature was kept within the normal range using a feedback-regulated heating pad. With an aseptic technique, the right vertebral artery was exposed in the lower neck and cannulated with a polyethylene catheter (0.86 mm). The dog was turned to the prone position, and an initial angiogram (pre-day 0) was obtained using 8 m*l* meglumine diatrizoate at a rate of approximately 3 m*l*/sec via a catheter inserted into the right vertebral artery. In the P-SAH and the sham groups, the occipital skin was surgically prepared, and a 4 cm skin incision was then made caudally starting from the external occipital protuberance. The occipital neck muscles were divided at the midline, and the atlanto-occipital membrane was exposed. Through a small incision made in the membrane, two silicon catheters were separately inserted into either side of the cerebellomedullary cisterns; one (1 mm in diameter, 5 cm long) was connected to an osmotic pump (Alzet Osmotic pump, Model 2ML1, ALZA Corporation, Palo Alto, CA, U.S.A.) placed subcutaneously to allow continuous administration of saline, while another was guided to the neck through the skin (1.2 mm in diameter, 8 cm long) for the intrathecal injection of autologous blood or saline. The tips of the catheters had several pores (0.5 mm in size) to facilitate the passage of injected materials. The length of the catheters in the

subarachnoid space was approximately 1.5 cm, which was sufficient to access the prepontine cistern. The incision of the atlanto-occipital membrane was closed with a surgical adhesive (alpha-cyano-acrylate monomer) (Sankyo Co., Ltd., Tokyo, Japan) to prevent cerebrospinal fluid leakage and blood invasion into the subarachnoid space. The cervical muscles and skin were then closed by suture. The osmotic pump contained 2 m*l* of saline. The pumping rate was 5×10^{-6} l/hr for 7 days (Fig. 1. A-B). In addition, one dog each of the P-SAH and the sham groups was administered 2 m*l* of dye (4% toluidine blue-saline) solution to examine the distribution of the treatment material using the osmotic pump.

Angiography was repeated to examine whether or not these procedures influenced the basilar artery diameter (post-day 0). After the control angiography, autologous non-heparinized fresh arterial blood (P-SAH group) or saline (sham group) (0.6 m*l*/kg body weight) was directly injected via the intrathecal catheter at a constant rate (1 m*l*/min) into the cerebellomedullary cistern, followed by removal of one-half of that volume of cerebrospinal fluid. The dogs were positioned with the neck flexed 30 degrees downward to facilitate distribution of the injection material throughout the entire prepontine cistern. After extubation, the dog was allowed to awaken. Forty-eight hours after the initial intrathecal injection, an additional blood or saline injection was given, as described above (day 2). Further angiograms were taken on days 2 and 7 in the same fashion. In the C-SAH group, SAH was produced according to the "two-hemorrhage" model described by Varsos *et al*. [18]. When all angiographical procedures had been conducted, the head of the animal was fixed at the same angle in a stereotaxic device under anesthesia, and the film focus distance was maintained thereafter at 60 cm. Therefore, magnification errors of the diameter size of the artery in the series of films are considered negligible in the same animal. All animals were treated with antibiotics after the surgical procedure, and other postoperative care included regular wound cleaning for 7 days.

The diameter of the basilar artery was measured at five corresponding locations along the vessel on the angiograms by means of an optical micrometer (OLYMPUS Optical Co., Ltd., Tokyo, Japan). All films were examined three times under blinded conditions by a single investigator. The sequential arterial changes are expressed as a percentage of the basilar artery diameter in the pre-day 0 angiogram in the same animal; and the accumulated angiography data from the respective groups are expressed as mean ± S.E. Differences between the calculated data at each time point were effected by Bartlett test and one way analysis of variance followed by Scheff and Dunnet multiple comparison procedures. A p-value smaller than 0.05 is considered to be statistically significant.

Dogs were sacrificed under sodium pentobarbital anesthesia (30 mg/kg, i.v.) and exsanguination after the final angiography on day 7. The basilar artery, including the brain stem, was fixed in 10% neutral buffered formalin,

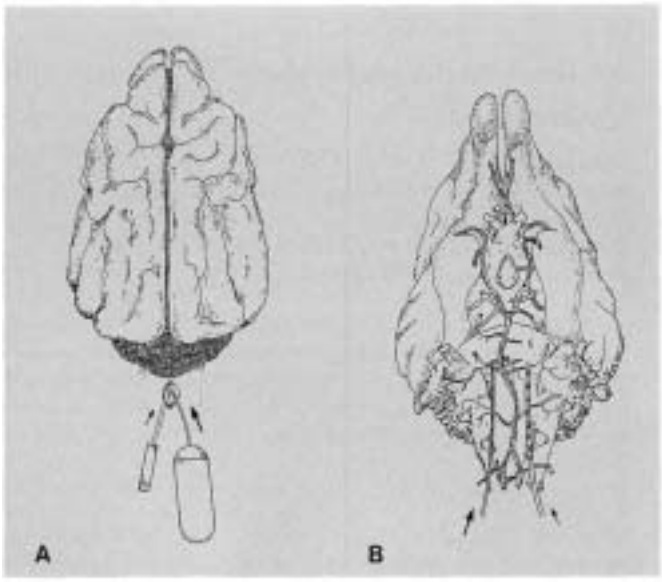

Fig. 1. A-B Schematic illustration of surgical procedure on the dorsal (A) and ventral surface (B) of the brain. Two intracisternal silicon catheters were inserted approximately 1.5 cm in the subarachnoid space through the atlanto-occipital membrane. One catheter (large arrow) was connected to an osmotic pump, and the other (small arrow) was used for the injection of autologous blood or mock treatment. The tip of the silicon catheters had several perforations (0.5 mm in size) specially made to facilitate a good liqid passage.

routinely embedded in paraffin, sectioned (3 μm) and stained with hematoxylin & eosin for light microscopical study.

In the P-SAH, the sham, and the C-SAH groups, the basilar artery diameter in the post-days 0 (the day of SAH being day 0) angiograms exhibited no significant difference [P-SAH group: 97.9 ± 1.1% (n=6), sham group: 98.4 ± 1.0% (n=4), C-SAH group: 98.7 ± 0.9% (n=6)]. The analysis of the repeated angiograms obtained on days 0 (post), 2, and 7 demonstrated that there was no significant change in the basilar artery diameter in the sham group [day 2: 9 7.0 ± 1.5% (n=4), day 7: 96.8 ± 1.2% (n=4)]. In contrast, the P-SAH and C-SAH groups exhibited a delayed and prolonged decrease in the basilar artery diameter. The basilar artery diameter was reduced to 63.1 ± 2.1% of the pre-day 0 value on day 2 and to 57.4 ± 2.7% on day 7 (P-SAH group: n=6, respectively), and to 76.5 ± 2.1% of the pre-day 0 value on day 2 and to 64.1 ± 2.7% on day 7 (C-SAH group: n=6, respectively) (p<0.01 *vs.* sham group, on days 2 and 7) (Fig. 2. A-D). In the P-SAH group, the basilar artery diameter was significantly decreased more than that of the C-SAH group (p<0.01 *vs.* C-SAH group, on days 2 and 7).

All dogs remained well and no focal neurological deficits developed during the course of the study. Upon postmortem examination, neither blockage or dislodgment of the intrathecal catheters, nor mechanical compression of the basilar artery was seen. In the dogs with dye injection, the dye administered via the osmotic pump was widely

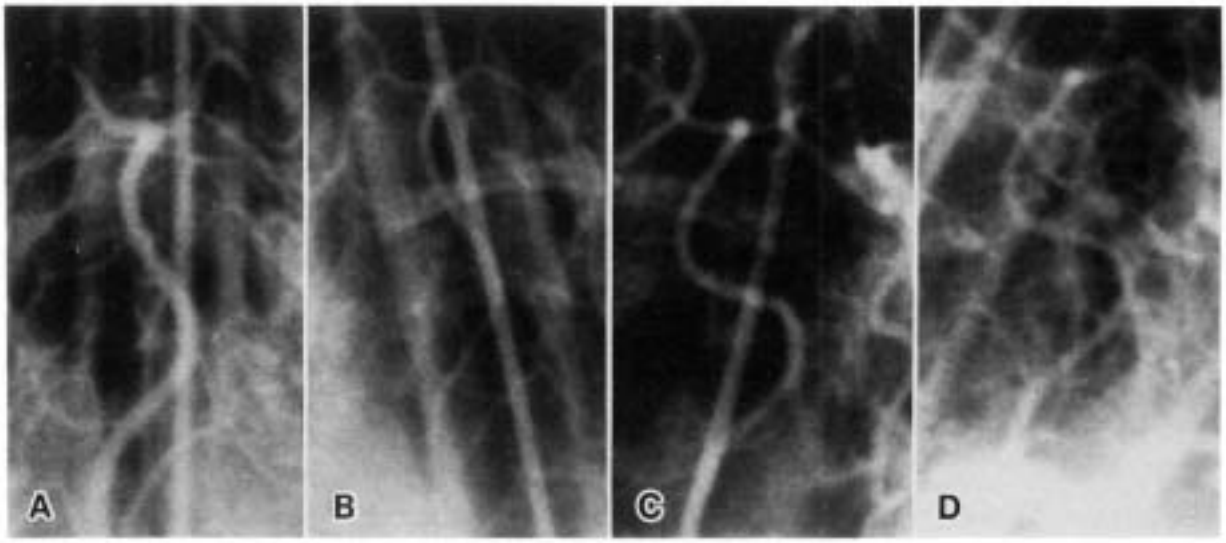

Fig. 2. A-D Angiogram of the basilar artery of a P-SAH group dog before the connection of an osmotic pump (pre-day 0) (A), and on day 7 (B) following the intrathecal injection of autologous blood. Severe angiographical narrowing of the basilar artery was observed on day 7. Angiogram of the basilar artery of a sham operation group dog before the connection of an osmotic pump (pre-day 0) (C), and on day 7 (D) following the intrathecal injection of saline. No angiographical changes of the basilar artery diameter were observed.

distributed in the prepontine cistern and the basal surface of the brain and brain stem. In the P-SAH group, a significantly larger blood clot was found in the basal cistern as compared to the C-SAH group. Upon histological examination, the subarachnoid space was found to have been expanded by numerous red blood cells. The accumulation of erythrophagocytic macrophages, neutrophils and lymphocytes around the spastic basilar artery and adjacent the smaller cerebral vessels indicated aseptic meningitis. The spastic vessels exhibited histological changes such as folding of the elastic lamina, detachment of rounded endothelial cells, and cytoplasmic vacuolation in the smooth muscle layer. In the sham group, no abnormal macroscopical or microscopical findings were observed in any animals.

Cerebral vasospasm is a major complication of human aneurysmal SAH gravely affecting outcome, but the underlying pathogenetic mechanism has remained unresolved and the treatment remains to be established. To investigate the pathophysiology and therapy of cerebral vasospasm, various animal models such as dogs [9–12, 18], monkeys [1, 6, 7], rabbits [2, 4, 15], cats [17], and rats [3, 8, 16] have been used. While each model has its merits and demerits, by far the most popular model has been the canine SAH model first proposed by Varsos *et al.* [18], designated the "two-hemorrhage" model. In this model, autologous non-heparinized arterial blood is injected into the cisterna magna twice at a 48-hr interval. Although species difference is a salient problem in cerebral vasospasm, the chronic cerebral vasospasm induced in this model has much in common with human cerebral vasospasm following aneurysmal SAH in terms of time course, response to pharmacological agents, and pathological changes in and around the cerebral artery.

While Varsos's "two-hemorrhage" model is thought to be the most reliable among those currently available, it requires repeated anesthesia and cisternal puncture procedure, that are not without problems. Repeated operative intervention under general anesthesia is not desirable in terms of animal protection. Cisternal puncture carried out in a blind fashion is not infrequently accompanied by technical hazards such as brain stem injury and faulty injection of blood, particularly on the second procedure. Furthermore, the fact that frequent cisternal puncture is harzadous makes the intrathecal administration of therapeutic agents difficult.

The placement of an intrathecally indwelling catheter has already been reported in a monkey SAH model [7], where the indwelling catheter was used solely for the purpose of intrathecal drug administration as SAH was induced by the placement of a blood clot around the middle cerebral artery by craniotomy. Since we hypothesized the use of such catheters not only for drug administration but also for the induction of repeated SAH might improve the canine "two-hemorrhage" model, we examined the feasibility of this technique in dogs in the present study.

The results show: i) the blood injected through the catheters effectively formed a large subarachnoid clot in the prepontine cistern; ii) the chronic cerebral vasospasm which invariably ensued tended to be more severe than those elicted in the previous canine models [9–12, 18]; iii) the fluid injected by the osmotic pump was evenly distributed in the cisterns around the brain stem; iv) no injury of the brain or the major arteries ascribable to the placement of catheters was found. Hence, this technique is considered to have advantages over those used to date in such points as i)

the repeated injection of blood is easy and sure; ii) the ensuing cerebral vasospasm tends to be severe; iii) since repeated anesthesia and operative interventions can be avoided, it is more favorable from the standpoint of animal protection; iv) chronic intrathecal administration of investigationed agents is possible. We believe, therefore, that the present model is useful for the investigation of the pathophysiology as well as the therapy of cerebral vasospasm, being more convenient and reliable than previous ones.

ACKNOWLEDGMENTS. This work was supported in part by grants-in-aid for scientific research (A) from the Ministry of Education, Science and Culture, Japan. We thank Kevin Boru, Ph.D., Chugai Pharmaceutical Co., Ltd. for review of the manuscript.

REFERENCES

1. Bevan, J. A., Bevan, R. M. D., and Frazee, J. G. 1988. *Stroke* 19: 73–79.
2. Endo, S., Branson, P. J., and Alksne, J. F. 1988. *Stroke* 19: 1420–1425.
3. Delgado, T. J., Brismar, J., and Svendgaard, N. A. 1985. *Stroke* 16: 595–601.
4. Fadel, M. M., Foley, P. L., Kassel, N. F., and Lee, K. S. 1995. *Surg. Neurol.* 43: 52–58.
5. Fisher, C. M., Kistler, J. P., and Davis, J. M. 1980. *Neurosurgery* 6: 1–9.
6. Handa, Y., Kabuto, M., Kobayashi, H., Kawano, H., Takeuchi, T., and Hayashi, M. 1991. *Neurosurgery* 28: 542–549.
7. Hino, A., Weir, B. K., Macdonald, R. L., Thisted, R. A., Kim, C. J., and Johns, L. M. 1995. *J. Neurosurg.* 83: 503–509.
8. Jackowski, A., Crockard, A., Burnstock, G., Russell, R. R., and Kristek, F. 1990. *J. Cereb. Blood Flow Metab.* 10: 835–849.
9. Kim, P., Schini, V. B., Sundt, T. M. Jr., and Vanhoutte, P. M. 1992. *Circ. Res.* 70: 248–256.
10. Kohno, K., Sakaki, S., Ohue, S., Kumon, Y., and Matsuoka, K. 1992. *Acta Neuropathol.* 81: 664–669.
11. Mori, T., Nagata, K., Ishida, T., Sasaki, T., Nirei, H., Hamada, K., Ohami, H., and Kirino, T. 1993. *J. Vet. Med. Sci.* 55: 581–586.
12. Peterson, J. W., Nishizawa, S., Hackett, J. D., and Zervas, N. T. 1990. *J. Neurosurg.* 72: 767–774.
13. Saito, I., Ueda, Y., and Sano, K. 1977. *J. Neurosurg.* 47: 412–429.
14. Sano, K. 1982. *Clin. Neurosurgery* 30: 13–58.
15. Shishido, T., Suzuki, R., Qian, L., and Hirakawa, K. 1994. *Stroke* 25: 864–868.
16. Solomon, R. A., Lovitz, R. L., Hegemann, M. T., Schnessler, G. B., Young, W. L., and Chien, S. 1987. *J. Cereb. Blood Flow Metab.* 7: 193–198.
17. Trojanowski, T. 1982. *Acta Neurochir. (Wien)* 62: 171–175.
18. Varsos, V. G., Liszczak, T. M., Han, D. H., Kistler, P., Vielma, J., Black, P. M., Hero, R. C., and Zervas, N. T. 1983. *J. Neurosurg.* 58: 11–17.