Comparison of three rat models of cerebral vasospasm

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Gules, Ilker, Motoyooshi Satoh, Ben R. Clower, Anil Nanda, and John H. Zhang. Comparison of three rat models of cerebral vasospasm. Am J Physiol Heart Circ Physiol 283: H2551–H2559, 2002; 10.1152/ajpheart.00616.2002.—A substantial number of rat models have been used to research subarachnoid hemorrhage-induced cerebral vasospasm; however, controversy exists regarding which method of selection is appropriate for this species. This study was designed to provide extensive information about the three most popular subarachnoid hemorrhage rat models: the endovascular puncture model, the single-hemorrhage model, and the double-hemorrhage model. In this study, the basilar artery and posterior communicating artery were chosen for histopathological examination and morphometric analysis. Both the endovascular puncture model and single-hemorrhage model developed significant degrees of vasospasm, which were less severe when compared with the double-hemorrhage model. The endovascular puncture model and double-hemorrhage model both developed more vasospasms in the posterior communicating artery than in the basilar artery. The endovascular puncture model has a markedly high mortality rate and high variability in bleeding volume. Overall, the present study showed that the double-hemorrhage model in rats is a more suitable tool with which to investigate mechanism and therapeutic approaches because it accurately correlates with the time courses for vasospasm in humans.

vasospasm; rat; models; subarachnoid hemorrhage

Prolonged Constriction of the cerebral arteries is the major cause of disability and death in patients with aneurysmal subarachnoid hemorrhage (1, 2, 14, 15, 21, 27). This is characterized by impairment of the dynamic balance between relaxing factors and contracting factors (10, 13, 23, 42, 48), which leads to cerebral arterial vasospasm. Approximately one-third of all patients with cerebral vasospasm develop delayed neurological ischemic deficits, which may resolve or progress to permanent cerebral infarction (37). Despite many years of intensive research, the mechanism for cerebral vasospasm has yet to be explained (25, 36).

An ideal animal model of subarachnoid hemorrhage-induced cerebral vasospasm must simulate arterial narrowing with related morphological changes and time courses relative to those in humans (4, 9, 29, 31). If this is not considered, it can lead us to inaccurate conclusions in the pathophysiology of cerebral vasospasm, meaning that the treatment efficacy in the clinical trial will not correlate with the efficacy in the clinical trial (35); therefore, the initial focus for cerebral vasospasm research is to choose an appropriate animal model of subarachnoid hemorrhage (31). A substantial number of studies on subarachnoid hemorrhage-induced cerebral vasospasm have been conducted. They used various species and various methods of inducing experimental subarachnoid hemorrhage such as intracisternal administration of blood (12, 19, 30, 38, 39, 43–46), endovascularly (5, 11, 22, 28, 38, 44, 47) or extravascularly (4, 20) puncturing the artery, and blood clot placement around the artery (15). It can be said that existing subarachnoid hemorrhage models of large animals, such as primates and canines, are the preferred models for cerebral vasospasm (31); however, these preferred models are high in cost and often limited in availability due to the difficulties involved in extensive surgery and handling of the subjects when inducing subarachnoid hemorrhage. In recent years, rats have become a popular species in the study of cerebral vasospasm for the following reasons: 1) all types of methods used to induce subarachnoid hemorrhage are possible in rats; 2) cerebral vasospasm after subarachnoid hemorrhage in rat species show biphasic patterns with early and late phases (12, 30, 44), as found in humans (24); 3) rat models display pathological alterations in major cerebral arteries (11, 19, 30) similar to that of humans (34); 4) it is possible to monitor physiological parameters such as mean arterial blood pressure, intracranial pressure, and cerebral blood flow in rats (4, 6, 20, 28, 30); 5) more information, such as genetic or genomic, is available in rats than in other large animals (7, 18); and 6) rat species also offer additional advantages in that they are available in large numbers, are inexpensive, and are easy to handle and care for.

To provide detailed, comprehensive knowledge of models for researchers in the future, we chose to compare three established rat models of subarachnoid

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hemorrhage-induced cerebral vasospasm in this study. The endovascular puncture model, single-hemorrhage model, and double-hemorrhage model were compared for mortality, degree of cerebral vasospasm, histological features, and time course of cerebral vasospasm.

MATERIALS AND METHODS

Experimental groups. The University of Mississippi Medical Center Committee on the Use and Care of Animals approved the protocol for the animals. Male Sprague-Dawley rats (n = 77) weighing from 300 to 350 g were divided into six groups. No surgery was performed on the first group, which was used as the control group (n = 10). In the second group, the single-hemorrhage model was induced by injecting 0.3 ml of nonheparinized fresh autologous arterial blood into the cisterna magna (n = 10). In the third group, the double-hemorrhage model was induced by a first injection of blood (0.5 ml), which was followed by a second injection of the same amount of blood 48 h later (n = 11). In the fourth group, the endovascular puncture model was achieved by puncturing the artery at the bifurcation of the internal carotid artery (ICA) (n = 25). In the fifth group, six rats were used in the single-hemorrhage model (n = 3) and endovascular puncture model groups (n = 3) and were euthanized on day 7 for the examination of cerebral vasospasm. Finally, in the seventh group, 15 rats were used for the observation of blood distribution 30 min after blood injection or arterial puncture.

Animals in the single-hemorrhage model and endovascular puncture model groups were euthanized on day 2 or 48 h after the blood injection or blood vessel puncture (except those in the fifth group). Animals in the double-hemorrhage model group were all euthanized on day 7 (Fig. 1). Each animal was anesthetized by an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg) and was allowed to breathe spontaneously. Animals were kept warm with a heating pad and were housed in a light-dark cycle environment with free access to food and water.

Animal models. The induction of subarachnoid hemorrhage using endovascular filament, the endovascular puncture model, has been described in detail (5, 47). Briefly, the rats were placed in the supine position. The ventral portion of the neck was shaved, and a midline incision was made. With the use of an operating microscope, we exposed the right external carotid artery and then ligated and transected the distal part of the external carotid artery, leaving a 3- to 4-mm stump. A 3-0 nylon filament was inserted into the external carotid artery and then advanced distally into the ICA until resistance was felt. Slight resistance was encountered at 22–25 mm from the bifurcation of the common carotid artery and then pushed further for perforation. After the suture withdrawal, the free stump of the external carotid artery was ligated using 3-0 silk sutures and the neck incision was closed.

The induction of subarachnoid hemorrhage using a single blood injection, the single-hemorrhage model, was induced by a posterior craniocervical approach (12). Shortly, after the animals were anesthetized, a small suboccipital incision was made, exposing the arch of the atlas, the occipital bone, and the atlantooccipital membrane. The cisterna magna was tapped using a 27-gauge needle, and 0.3 ml of cerebral spinal fluid were gently aspirated. Freshly drawn blood (0.3 ml) from the femoral artery was then injected aseptically into the cisterna magna over a period of 2 min. Immediately after the injection of blood, the hole was sealed with glue to prevent further blood loss. To permit blood distribution around the basal arteries, the animal was tilted at 20° for 30 min with the head lowered. The animal recovered from the effects of anesthesia and was returned to its cage.

The induction of subarachnoid hemorrhage using a double injection, the double-hemorrhage model, was caused by repeating a second blood injection 48 h after the first induction of subarachnoid hemorrhage using the same method (30, 44).

The rats were reanesthetized and euthanized 48 h after the induction of subarachnoid hemorrhage in the single-hemorrhage model and endovascular puncture model groups and on day 7 in the single-hemorrhage model, endovascular puncture model, and double-hemorrhage model groups. By means of transthoracic cannulation of the left ventricle, they were perfused with 300 ml of phosphate-buffered saline solution and reperfused with a mixture of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) under a pressure of 120 cmH2O. We chose to study the basilar artery and posterior communicating artery for histopathological examination and morphometric analysis. The rats’ brains were immediately removed and placed in the same fixative solution for 24 h.

In five additional animals in each group, we also examined the distribution of subarachnoid clots. This was done 30 min after the intracisternal injection of blood in the single-hemorrhage model group, 30 min after the artery was punctured in the endovascular puncture model group, and 30 min after the second intracisternal injection of blood in the double-hemorrhage model group. The same examination of blood distribution was repeated 48 h after subarachnoid hemorrhage in the single-hemorrhage model and endovascular puncture model groups, and it was repeated on day 7 for the animals in the double-hemorrhage group whose experimental procedure had been completed.

Transmission electron microscopy and morphometric studies. For transmission electron microscopy (TEM) studies, the arteries were placed in buffered 1% osmium tetroxide, dehydrated in graded ethanol, embedded in epon-araldite epoxy resin, sectioned at 90 Å, and examined by a LEO 906 TEM. For morphometric measurements, the luminal perimeter and wall thickness of the arteries in each specimen were measured using a digitized image analysis system with morph software (7, 33). The specimens for the light microscope study were dehydrated in graded ethanol, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.
Light microscopic sections of arteries were projected as digitized video images. The inner perimeters of the vessels were measured by tracing the luminal surface of the intima. The thickness of the vessel wall was determined by taking four measurements of each artery that extended from the luminal surface of the intima to the outer limit of the media, so as not to include the adventitia (30). Those four measurements were averaged for one score. An independent investigator took the measurements.

Statistical analysis. All values are expressed as means ± SE. Statistical differences between the groups were compared using one-way ANOVA and the Bonferroni test. Any probabilities less than 5% (P value < 0.05) were considered to be significant.

RESULTS

Mortality. No mortality due to subarachnoid hemorrhage was observed in the single-hemorrhage model group, 1 occurrence (9%, 1 of 11 rats) was observed in the double-hemorrhage model group, and 13 occurrences (56.7%, 13 of 25 rats) were observed in the endovascular puncture model group (Fig. 2). The one occurrence of mortality in the double-hemorrhage model group was encountered on the third day after the second injection. There was markedly high mortality in the endovascular puncture model group. Animals that died in the endovascular puncture model group either did not recover from the effects of the anesthesia or displayed severe lethargy and breathing trouble until they died. In the endovascular puncture model group, four incidences of mortality occurred within the first 6 h, six occurred within 6–24 h, and three occurred within 24–48 h after subarachnoid hemorrhage. Two animals failed to show subarachnoid hemorrhage after puncture and were excluded from the statistical analysis.

From the animals that completed the experimental period (48 h in the single-hemorrhage model and endovascular puncture model and 7 days in the single-hemorrhage model, endovascular puncture model, and double-hemorrhage model), no neurological deficits were observed after the induction of subarachnoid hemorrhage. Lethargy and decreased appetite were encountered in all models in acute terms of subarachnoid hemorrhage, but lasted longer in the endovascular puncture model group.

Blood distribution after subarachnoid hemorrhage. In acute terms at 30 min after blood injection or blood vessel puncture, on gross inspection, the widespread distribution of blood was seen in the basal cisterns, on the frontoorbital surface, over the cerebral convexities, and rarely in the lateral ventricle in all models. The animals in the endovascular puncture model group, however, showed more blood on the frontoorbital surface and on the cortical surfaces and much less blood in the cisterna magna than the other groups. Capping clots were frequently encountered at the point of perforation in the endovascular puncture model group, and there was variability with respect to the amount of blood. At the necropsy of the animals that died after being punctured the first day, excessive blood was found in the cisterns. Also, five animals in the endovascular puncture model group showed intracerebral hemorrhages and severe subdural hemorrhages in addition to subarachnoid hemorrhage.

In the chronic term, none of the models developed blood clotting around the basilar artery at the time of death (48 h in the single-hemorrhage model and endovascular puncture model and 7 days in the double-hemorrhage model), although the existence of dotted blood clots around the basilar artery was noticed in all subarachnoid hemorrhage groups. The posterior communicating artery was surrounded by xanthochromic discoloration. The appearance of this in both arteries was more distinct in the double-hemorrhage model group. In the animals of injected groups (single-hemorrhage model and double-hemorrhage model), there were always dense blood clots over the cerebellar surface on day 2. A few dotted blood clots were observed on day 7 in rats of either the single-hemorrhage model or endovascular puncture model. The control group of the animals (no blood injection or blood vessel puncture) exhibited no blood in the subarachnoid spaces.

Morphometric vasospasm. Figure 3 shows how we measured the inner perimeter and arterial wall thickness. In the three types of subarachnoid hemorrhage models, both the basilar artery and posterior communicating artery revealed luminal narrowing and increased wall thickness. The mean perimeter was reduced from control levels in the basilar artery by 33.33% in the double-hemorrhage model group, 20.18% in the single-hemorrhage model group, and 16.5% in the endovascular puncture model group. The mean perimeter was reduced from control levels in the posterior communicating artery by 34.73% in the double-hemorrhage model group, 20.3% in the single-hemorrhage model group, and 24.55% in the endovascular puncture model group (Figs. 4 and 5). Compared with the control group, all subarachnoid hemorrhage models showed significant reduction in the luminal perimeter of the basilar artery and posterior communicating artery (P < 0.05, ANOVA). When subarachnoid hemorrhage models were compared with each other, the double-hemorrhage model group showed a significantly
higher (% < 0.05) effect on the basilar artery than the other models. The effects on the posterior communicating artery in the double-hemorrhage model group were significantly higher than in the single-hemorrhage model group (% < 0.05) only. Interestingly, the endovascular puncture model group exhibited more contractile effects on the posterior communicating artery (24.55%) than on the basilar artery (16.5%).

The subarachnoid hemorrhage-induced increases in wall thickness of the basilar artery were 69.38% in the double-hemorrhage model group, 61.95% in the single-hemorrhage model group, and 52.60% in the endovascular perforation model group. The increases in wall thickness of the posterior communicating artery were 95.06% in the double-hemorrhage model group, 62.01% in the single-hemorrhage model group, and 72.66% in the endovascular perforation model group (Figs. 4 and 5). Compared with the control group, all subarachnoid hemorrhage models showed a significant increase (% < 0.05, ANOVA) in wall thickness of the basilar artery and posterior communicating artery. In a comparison of subarachnoid hemorrhage models to each other, the double-hemorrhage model group exhibited a more significant effect (% < 0.05) on the basilar artery than in the single-hemorrhage model group. There were no significant differences (% > 0.05) among the three subarachnoid hemorrhage models for wall thickness in the posterior communicating artery.

In rats that were euthanized on day 7 from the single-hemorrhage model group and endovascular puncture model groups, no apparent vasospasm was observed and the data were not calculated.

Histopathological examination. Figure 6 shows light microscopic pictures of arteries from the control group and subarachnoid hemorrhage models. Different degrees of vasospasm were observed in all models of subarachnoid hemorrhage.

The ultrastructural micrograms of the basilar artery and posterior communicating artery were examined in the control group of animals (Fig. 7A). There were similar findings found in that the endothelial cells were flat in shape, tightly attached to the internal elastic lamina, and characterized by a single continuous layer of contacts of varying length with tight junctions and occasional indentations. There was no vacuolization in either endothelial or smooth muscle cells.

Pathological changes were observed mainly in the endothelial cells of the basilar artery and posterior communicating artery. These changes include swelling, rounded shape, vacuolizations in the cytoplasm and nuclei, disruption of tight junctions, and widening of interendothelial spaces in all of the subarachnoid hemorrhage models (Fig. 7, A and B). Additionally, detachment of endothelial cells and, in rare instances, necrosis was encountered in the double-hemorrhage model group. It was also found that the internal elastic lamina was corrugated and contained increased thickness. The changes described above, observed in the single-hemorrhage model and endovascular perforation model groups, are similar in degree with the exception of the posterior communicating artery. Interestingly, both the endovascular perforation model and double-hemorrhage model groups produced more pathological changes in the posterior communicating artery; however, the pathological findings appeared more severe, extensive, and consistent in the double-hemorrhage model group than in the other groups.

DISCUSSION

In the history of experimental subarachnoid hemorrhage research, the first use of a dog model was reported in 1928 (32); however, the rat model has a much shorter history, ~20 yr, in this area of research (4). Our current knowledge of experimental subarachnoid hemorrhage in rat models is mostly limited to the single-hemorrhage model (11, 12, 19). Only recently have the double-hemorrhage model (30, 44) or endovascular puncture model (38) been reported on. Furthermore, most of the studies that used the endovascular puncture model in the rat were employed to investigate the pathophysiology of subarachnoid hemorrhage (22, 28) rather than cerebral vasospasm itself (38). We feel that there is an urgent need to compare the rat models of subarachnoid hemorrhage-induced cerebral vasospasm and to establish a guideline for future research.

It is believed that the rat species is a poor model for subarachnoid hemorrhage-induced cerebral vasospasm
for many reasons. First, there are documented anatomic differences between the cerebral arteries found in rats and those found in humans. For instance, there is a difference in wall structure (17) as well as the abundance of collateral blood flow (20). Also, the lack of mural interadventitial spaces in the rat can make this species more resistant to cerebral arterial constriction. The obstruction of these spaces by blood clots in humans results in a decrease in nourishment to the arterial wall, which may be partly responsible for vasospasm (8, 41); however, subarachnoid hemorrhage rat models can exhibit degrees of cerebral vasospasm (12, 30, 44) in a biphasic manner as relative to humans. These responses to subarachnoid hemorrhage are either mild or moderate depending on the chosen model (24, 35). Another reason the rat species is believed to be a poor model is that ischemic consequences after subarachnoid hemorrhage cannot be obtained in a rat model because of the above-mentioned anatomic difference (20). It should, however, be remembered that vasospasm-related ischemic deficits cannot be observed even in a primate model (15, 31). The usability of angiogram in rats to estimate the extent of the induced vasospasm can be another problem (12). It is difficult due to the small size of the animal, especially when a repetitive angiographic procedure is needed; nevertheless, it is possible. In addition to angiographic evaluation, morphometric assessment is used exten-

Fig. 4. Graphs showing changes in luminal perimeter and wall thickness of the basilar artery (BA) in the control group and SAH models. Animal number is the same (n = 10) for each group. A: perimeter of vessels. Note the significant decrease in luminal perimeter in all SAH groups compared with the control group. The DHM showed significant differences compared with other SAH models. B: wall thickness of vessels. Note the corresponding increase in wall thickness to luminal perimeter in all SAH models compared with the control group. The DHM showed significant differences only from the EPM. Morphometric analyses for the BA were performed 48 h after the induction of SAH in the SHM and EPM groups and on day 7 in the DHM group. Values are means ± SE. *P < 0.01; †P < 0.05.

Fig. 5. Graphs showing changes in luminal perimeter and wall thickness of the posterior communicating artery (PCommA) in the control group and SAH models. Animal number is the same (n = 10) for each group. A: perimeters of vessels. Note the significant decrease in luminal perimeter in all SAH groups compared with the control group. The DHM showed significant differences from the SHM. B: wall thickness of vessels. Note corresponding increase in wall thickness to luminal perimeter in all SAH models compared with the control group. There were no significant differences among the three SAH models. Values are means ± SE. *P < 0.01; †P < 0.05, respectively. Morphometric analyses for the PCommA were performed 48 h after the induction of SAH in the SHM and EPM groups and on day 7 in the DHM group.
sively in experimental research. In recent years, the rat species has regained popularity for subarachnoid hemorrhage research after undergoing several technical modifications (44, 47).

Considering the natural occurrence mechanism of subarachnoid hemorrhage and the location of bleeding in humans with ruptured aneurysms, of which ~85% are located in the anterior circulation (21), the endovascular puncture model has more similarities to humans (39, 47). On the other hand, intracisternal injection methods (either single or double hemorrhage) neglect injury of the artery, which may have potentially harmful effects on cerebral vasospasm (47). Although similar complex pathophysiological phenomena after the induction of subarachnoid hemorrhage have been documented in all models, the endovascular puncture model showed some distinctions from that of the single-hemorrhage model, such as relatively long-lasting, high intracranial pressure and reduced cerebral blood flow values in the acute stages of subarachnoid hemorrhage (39, 40, 47). It was also reported that intracranial pressure monitoring via the cisterna magna in the endovascular puncture model is more reliable because it lacks additional intervention, like that which occurs in the single-hemorrhage model or double-hemorrhage model (39, 40); however, the correlation among intracranial pressure, cerebral blood flow changes and vasospasm are not clear (5, 6, 37). In contrast with the simplicity of subarachnoid hemorrhage-induction in injection models, the microsurgical approach required in the endovascular puncture model is much more complicated and extensive and requires more time than the others. The problem with the length of time may eventually be resolved with experience. Unfortunately, it is difficult to say whether this is true for the difficulties encountered in surgical technique and technical requirements.

Existing literature documents that high mortality rates for the endovascular puncture model, up to 50% in the first 24 h, have been reported (5). The data presented in the current study demonstrated a 56.5% mortality rate within the first 7 days. No mortality was encountered in the single-hemorrhage model, and only 9% was encountered in the double-hemorrhage model. The source most likely responsible for mortality, especially in the endovascular puncture model, is probably irreversible brain damage. It may be associated with the high volume of blood and its uncontrollable jet flow through injured arteries into the brain and subsequently related pathophysiological changes (5). Another possibility may be multiple bleedings from injured arteries (31). On the other hand, rapidity of blood injection can be easily controlled in injection models, and there are no unexpected rebleeding risks (30). Several authors have redesigned the surgical procedure in the endovascular puncture model to control the volume as well as the speed of bleeding into cisterns. They have tried either obliterating the carotid artery (22, 47) or decreasing the diameter of the suture used to puncture the artery (39). Although those practices have demonstrated positive results in reducing mortality in studies, the authors pointed out that it can result in cerebral ischemia or decreased blood volume in cisterns (39, 40, 47). Brain damage in the endovascular puncture model is not limited to ischemia, which can be combined with direct insult of suture to the brain tissue (47). This may cause the release of additional mediators from a damaged brain and arteries may reveal varying physiological responses. Another problem with the endovascular puncture model is failure of induction of subarachnoid hemorrhage, as observed in our study and in others, even with use of intracranial pressure monitorization (47). This may present the need for a greater number of experimental animals for the endovascular puncture model. The unavailability of a vehicle group for research on the role of blood in the pathophysiology in the endovascular puncture model is another area of concern (5, 47).
Fig. 7. Transmission electron micrographs of the BA (A) and PCommA (E) from the control group. The endothelium is flat and tightly attached to the IEL. The IEL is thin and nonconvoluted. The BA in the EPM and SHM (B and C) showed similar histopathological changes, such as atypically shaped and vacuolated endothelium and increased thickness of the IEL. Samples from the DHM (D) showed similar shape, detachment of the endothelium, corrugation of the IEL with increased thickness, and, rarely, necrosis (arrow). The PCommA in all SAH models (F–H) showed similar pathological findings, such as atypically shaped and vacuolated endothelium and corrugation of the IEL with increased thickness, but those changes occurred more severely in the DHM. Scale bar = 0.002 mm; L = lumen.
In the production of vasospasm, the most important factor, as shown in both experimental (12, 26, 43, 44, 46) and clinical studies (16, 29), is the amount of blood in contact with cerebral arteries. Rats are unable to maintain an adequate amount of periarterial clotting because of the rapid clearance time, which usually occurs 48 h after subarachnoid hemorrhage induction (12, 19, 30). The result of the present study is consistent with previous reports. None of the three models could retain the blood clot around the basilar artery until the time of death (48 h after subarachnoid hemorrhage in the single-hemorrhage model and endovascular puncture model and 7 days after subarachnoid hemorrhage in the double-hemorrhage model), although the existence of dotty-fashioned blood was noticed. Despite the fact that all subarachnoid hemorrhage models in the present study developed sufficient vasospasm, the most severe arterial contraction in both arteries was found after induction in the double-hemorrhage model on day 7. Similar results have been previously reported: that the single injection of blood produced less vasospasm, especially on day 7, and a second one (double hemorrhage) was required to produce a more reliable vasospasm model in rabbits and dogs (26, 43, 46).

Another important problem is the discrepancy in the time course of vasospasm between rats and humans. Maximum angiographic vasospasm appears between 6 and 8 days after subarachnoid hemorrhage in humans (3). The single-hemorrhage model (12) and endovascular puncture model (38) show maximum narrowing at day 2, whereas the double-hemorrhage model (30, 44) has the same time course as humans, with maximum narrowing at day 7. Even though cerebral vasospasm was observed in both the basilar artery and posterior communicating artery on day 7 in the double-hemorrhage model, vasoconstriction tends to be more severe in the posterior communicating artery than in the basilar artery. In the present study, vasoconstriction was more severe in the posterior communicating artery, which was more severe in the double-hemorrhage model group. The changes were more consistent and severe in the double-hemorrhage model group.

In conclusion, all rat models of subarachnoid hemorrhage offer reliable and reproducible vasospasm-containing histopathological evidences. Both the single-hemorrhage model and endovascular puncture model revealed equal results for vasospasm except in the posterior communicating artery, which was more severe in the endovascular puncture model. The severity was equal to the severity observed in the double-hemorrhage model. The endovascular puncture model displayed an unacceptable mortality rate. In addition, the existence of varying degrees of cerebral insult can also limit the use of this model. As a result, the double-hemorrhage model proved to be effective in producing a higher degree of vasospasm with lower mortality. In our opinion, it seems more reasonable to use the double-hemorrhage model as a subarachnoid hemorrhage model.

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