Small Animal Model for Investigation of Subarachnoid Hemorrhage and Cerebral Vasospasm

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SUMMARY A method for induction of subarachnoid hemorrhage (SAH) in a rat model is described. Resolution of the hemorrhage was documented photographically and microscopically at intervals from 1 hr to 8 days. Photographs indicated that most of the hemorrhage was resorbed within 3 days, an observation confirmed microscopically by the amount of red blood cells in the subarachnoid space. Significant cerebral vasospasm was documented within the first 2 days after the induction of hemorrhage with the basilar artery returning to baseline values at an average of 3 days followed by moderate dilatation at 5 to 8 days. The suitability of the rat as an animal model for further investigation of subarachnoid hemorrhage is discussed.

INVESTIGATION of subarachnoid hemorrhage (SAH) and cerebral vasospasm has centered on a number of factors including: 1) mechanical, 2) histological, and 3) pharmacological. Several animal species have been utilized for study including dogs, cats, and monkeys. The possibility of using a smaller, less expensive laboratory animal has not been thoroughly investigated. The present investigation sought to determine whether the rat was a suitable animal for the study of SAH with regard to the mode of SAH induction, its time course of resolution and the degree of cerebral vasospasm produced.

Materials and Methods

All animals were 400–600 g male Sprague-Dawley rats. They were anesthetized with intraperitoneal injections of sodium nembutal (40–50 mg/kg). The rats were placed in the supine position, the ventral portion of the neck shaved, and a midline incision approximately 2.5 cm in length made. The trachea was exposed and an endotracheal tube (ID 0.062", OD 0.082") inserted orally, and visualized entering the exposed trachea. Repeated suction ensured maintenance of an adequate airway. Using an operating microscope, the cervical musculature and carotid arteries were retracted to expose a 15–20 mm² area of clivus. A midline craniectomy (less than 2 mm²) was performed using a #23A Horico diamond burr to expose the normally transparent dura and underlying basilar artery. A tungsten microelectrode, drawn to a 40–60 micron tip, was positioned over the exposed basilar artery using a stereotactic device (fig. 1). The tip was advanced through the intact dural and arachnoid membranes and into the lumen of the basilar artery. SAH was induced upon slow withdrawal of the tungsten tip. The subarachnoid progression of the hemorrhage was visualized microscopically through the craniectomy. Gelfoam® (absorbable gelatin sponge, The Upjohn Company, Kalamazoo, MI) was placed at the craniectomy site and the neck incision closed with skin clips. The endotracheal tube was left in place approximately 1–2 hr postoperatively until the animal had recovered from the effects of the anesthesia and was without obvious respiratory distress.

The site of the SAH was reexposed 1 hr (acute), or 1, 2, 3, 5, and 8 days postoperatively. Animals used acutely were maintained on sodium nembutal postoperatively. After recovering from initial surgery, all chronic animals were reanesthetized, trachotomized, and intubated. The clivus was reexposed and the craniectomy enlarged with the diamond burr revealing a larger segment of the basilar artery (approximately 7–10 mm). The dura was then carefully removed allowing better visualization of the underlying basilar artery and brainstem. Photographs were taken through a sidearm extension of the operating microscope at 25X using a Nikon camera and Kodak Tri-X (400 ASA) film. A beam-splitter which directed 70% of the light to the camera was utilized. Photographs were also taken of control (no SAH) basilar arteries. Photographs were enlarged and the area of similar segments of the basilar artery in mm² was determined by the use of a planimeter. Changes in area at the various time points were expressed as a percentage of the control values.

Rats were sacrificed by left ventricular perfusion with gluteraldehyde-phosphate buffer (pH 7.4). The brains were removed and stored in gluteraldehyde-phosphate buffer for 24 hours prior to being placed in 5% sucrose-phosphate buffer (pH 7.4). Brain stem segments containing the basilar artery were cut into 1 mm sections, postfixed in 2% osmic acid in phosphate buffer (pH 7.0), dehydrated through a series of graded ethanol, and embedded in epon resin. The embedded tissue was cut into 1 micron sections for light microscopy on a Sorvall MT2 microtome and stained with 1% toluidine blue in 1% sodium borate.

Results

Overall mortality from the procedure in our series of 74 animals was 26%, the majority of the deaths occurring between 1 and 48 hr. The greatest source of difficulty were respiratory problems. Animals did not display any obvious signs of paralysis and the major
MODEL FOR SAH AND VASOSPASM/Barry, Gogian, Stein 539

of surviving animals regained normal activity and feeding habits by 3 days after surgery.

Photographs of basilar arteries taken at various time points are shown in figure 2 (A-G). These photographs show the time course of the resolution of the SAH and indicate that most of the hemorrhage had been resorbed within 72 hr. Trace hemorrhagic staining of the brain stem adjacent to the basilar artery was found up to 8 days in all the surviving animals.

Light microscopic photographs taken at various time points are shown in figure 2 (H-N), and indicate a similar time course of the resolution of the SAH as gauged by the amount of red blood cells in the subarachnoid space.

Photographs of in situ basilar arteries similar to those in figure 2 (A-G) were also used to determine the degree of basilar vasospasm present at a particular time. Only those arteries at each time point which had not been subjected to inadvertent trauma during reexposure, e.g. mechanical stimulation, or rupture of a subarachnoid vessel, were photographed. The results indicated a significant degree of spasm of the artery up to 2 days ($p < 0.05$), no significant difference from control at 3 days, and a significant dilatation at 5 to 8 days (figure 3).

Discussion

Previous investigations of SAH have utilized autologous blood introduced into the subarachnoid space, cutting arteries in the subarachnoid space, and direct puncture of vessels with 30-gauge needles to simulate SAH. Our technique differed in that SAH was induced by creation of a lesion in the wall of the basilar artery with a stereotactically controlled tungsten needle thereby emulating the natural occurrence of SAH by rupture of an aneurysm. It permitted a controlled bleeding which could be visualized with the aid of an operating microscope spreading into the subarachnoid space over the surface of the brain stem.

The overall mortality of 26% in this series may be explained by a number of operative technique factors thereby making it difficult to assign mortality to any one. The most likely sources were possible irreversible brain damage from the lesion, respiratory complications from intubation and inability to clear secretions, and other factors such as intolerance of the usual dose of anesthetic, trauma to vital structures in the operative field and postoperative infection. Taking these factors into account, the overall mortality of 26% in this study is acceptable.

A review of the literature has failed to reveal previous animal studies on the time course for resolution of induced SAH. Our investigation indicated nearly total resolution of the hemorrhage by 3 days post-SAH, and only peripheral hemorrhagic staining of the brain stem adjacent to the basilar artery thereafter. These results correlated with the degree of spasm of the basilar artery which was most marked between 1 and 48 hr and back to control values by 72 hr. The apparent dilatation of the artery 5 to 8 days after the induction of SAH is difficult to explain.

The work of Brawley et al. in dogs, demonstrated that spasm is a "biphasic" phenomenon consisting of an acute phase lasting about 1 hr, followed by a chronic phase occurring 3 days post hemorrhage and persisting for days thereafter. This "biphasic" course of spasm has also been reported in monkeys. The present results did not demonstrate a biphasic time course of cerebral vasospasm in the rat at the intervals chosen to examine the artery, but rather showed only an immediate onset of spasm which persisted for 2-3 days followed by a moderate dilatation. It is possible that this biphasic course can be observed in the rat by using an interval between time points longer than the ones used in this study. The duration of vasospasm, however, followed closely the time course of the resorption of the subarachnoid blood clot. It has been shown that vasoactive agents are released during the fibrinolysis of subarachnoid blood and it has been suggested that the time course of prolonged experimental vasospasm coincides with the progressive release of a vasospasmogenic substance from blood in the subarachnoid space. It is possible that in the rat the rate...
of resolution of the hemorrhage limits the availability of vasospasmogenic substances which may contribute to a prolonged phase of cerebral vasospasm. The original intent of this research was to determine the suitability of the rat as a model for the study of SAH. As with any animal model there are deficiencies. In the rat, because of potential trauma from repeated exposure of the basilar artery, data can only be obtained from one reexposure in each animal. A second deficiency is the open skull which nullifies any effect that changes in intracranial pressure would have on spasm. Despite these deficiencies in the model the relative simplicity of the surgical procedures and the similarity of the induction of the lesion to the natural occurrence of SAH, combined with the low cost and availability of this animal, make it a useful model. Unlike other investigators utilizing conventional arteriography for the documentation of spasm, the photographic techniques employed in this study permitted direct observation of the resolution of the hemorrhage and resultant vasospasm. Future investigation using the rat as a model for SAH is warranted on the basis of the reported results.

References
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