Heparin reduces proliferative angiopathy following subarachnoid hemorrhage in cats

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Subarachnoid hemorrhage (SAH) was produced in cats by transorbital rupture of the right middle cerebral artery (MCA). In untreated cats, widespread proliferative angiopathy occurred in both MCA's by 16 days after SAH. In cats that received systemic heparin, the pathological events following SAH were clearly reduced in the ruptured artery, and were not present in the contralateral left MCA. Platelets are known to adhere to the subintimal surface of cerebral arteries after SAH. The authors suggest that platelet-derived growth factor released from the intimal platelet carpet following SAH may be the stimulus for the development of proliferative angiopathy, and that this platelet factor is inhibited by heparin.

KEY WORDS • subarachnoid hemorrhage • systemic heparin • proliferative angiopathy • vasospasm

SYMPTOMATIC clinical vasospasm occurs in approximately 20% to 30% of patients following subarachnoid hemorrhage (SAH), and the resulting neurological deficits are the most frequent cause of delayed morbidity in these patients. In 1982, we reported the outcome in 112 patients with ruptured intracranial aneurysms treated with systemic heparin during gradual carotid ligation. In these patients, the incidence of ischemic neurological deficits that occurred between clamp application and hospital discharge was significantly reduced when compared to a comparable retrospective series of control patients derived from the results of the Cooperative Study.

To explain the reduction in ischemia in heparinized patients, we proposed a triphasic concept of the pathophysiology of cerebral ischemia following SAH. This concept assumes a sequential interrelationship between the multiple changes in the cerebral arteries following SAH which have been described by various investigators and may serve as a unifying concept in the understanding of this complex phenomenon. We proposed that the initial muscular contraction of the arterial wall, a nonspecific response to a variety of stimuli, is followed by a second phase which consists of detachment of arterial endothelial cells and the formation of an intimal platelet carpet. The third phase is resolution of the platelet carpet, associated with the migration and proliferation of cellular elements into the subendothelial zone of the arterial wall, producing structural stenosis and loss of vessel compliance.

The present study was performed to examine the effect of heparin on the changes that develop in the intracranial arteries following SAH. Further, if anatomical changes significantly contribute to the radiographic appearance of vessel constriction following SAH, correlated anatomical events within a large segment of the cerebral vascular tree become of paramount importance. Therefore, the propagation of pathological changes throughout a large segment of the cerebral vascular network was also examined.

Materials and Methods

Thirty-two adult cats were divided into three groups. The method of producing SAH has been described previously, and is based upon the rupture of the right middle cerebral artery (MCA). These studies are in compliance with the American Hospital Association humane guidelines and further approved by the local University Medical Center Animal Review Committee. Under general anesthesia with intramuscular ketamine (20 mg/kg), the animal's head was immobilized in a stereotaxic instrument. After orbital exenteration, a small craniectomy was performed adjacent to the right
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optic foramen, exposing the right MCA. A suture-hook made from a sharpened No. 27 needle was passed through the wall of the vessel. The dura was closed within the orbit. Following a 7-day healing period, cats were anesthetized again and the hook was pulled from the vessel wall by means of the suture that had been left in the subcutaneous tissue of the orbit, resulting in a "closed space" SAH. To prevent wound infection, Keflin (cephalothin, 50 mg/kg/day) was administered intramuscularly for 5 days following the initial suture-hook operation.

Heparin was administered as follows. Cats received 75 mg/kg heparin subcutaneously every 6 hours, beginning 6 hours after the initial placement of the suture-hook into the vessel wall. Heparin was discontinued 12 hours before pulling the suture-hook from the vessel wall (induced SAH), and was resumed 6 hours after induced SAH and continued until the cats were sacrificed 16 days post-SAH. This 16-day delay between SAH and sacrifice was chosen because previous studies have shown that advanced vessel pathology has occurred by this time. In Group I (three cats) served as controls for establishing the normal architecture of the MCA in cats. In Group II (17 cats), all cats were sacrificed 16 days after induced SAH. These animals did not receive heparin. In Group III (12 cats), the animals were treated with heparin (as described above) and then sacrificed 16 days following SAH.

For light microscopic study, brains were perfused with cold saline and 10% formalin, then removed from the cranial cavity. Both MCA’s and the basilar artery were dissected from their beds. Vessels were fixed in 10% formalin and embedded in paraffin. Arterial cross-sections (6 μ thick) were stained with hematoxylin and eosin. Serial sections of whole brains (25 μ thick) were stained with thionine and by Prussian blue techniques.

In order to correlate variations in pathological events among the experimental groups, a method of grading became necessary. The present pathological grading system has been described previously, and is based primarily upon the severity of intimal proliferation, and other complementary changes. The earliest changes (Grade I changes) consisted of mild intimal proliferation of both endothelial cells and smooth-muscle cells two to three cell layers thick; there was also mild splitting and corrugation of the internal elastic membrane, myonecrosis, and intramural hemorrhage. Grade II arteriopathy consisted primarily of intimal proliferation, three to eight cell layers thick, corrugation of the internal elastic membrane, and myofibrosis. Grade III arteriopathy included the most severe vessel changes, consisting of a combination of severe intimal proliferation greater than eight cells thick, myofibrosis, corrugation and disruption of the internal elastic membrane, and severe shrinkage of the media of the vessel.

In order to evaluate the longitudinal spread of morphological changes, the following procedures were performed on both MCA’s of cats in both experimental and control groups. Light microscopy studies of MCA

**Fig. 1.** Photomicrograph of the right middle cerebral artery from a control cat showing a single layer of endothelial cells along the intima of the vessel (arrows). H & E, x 280.

cross-sections (6 μ thick) were performed in a proximal-distal direction. The first and last appearance of vessel damage was noted, and the number of serial sections was multiplied by six and converted to millimeters to allow approximation of spread of damage. Diagrams to show the propagated proliferative changes were then made.

**Results**

**Survival after Subarachnoid Hemorrhage**

Group II initially contained 17 untreated cats with SAH. Following SAH, the cats tended to ignore food and water for a few days, and left hemiparesis developed immediately in two. This condition remained unchanged in one cat, while the other cat gradually recovered over an 8-day period. Of the 17 cats exposed to induced SAH, only 10 survived for 16 days (59%). The other seven died 3 to 5 days following SAH. Group II initially contained 12 cats treated with heparin and subjected to SAH. These also tended to ignore food and water for a few days post-SAH. One cat developed left hemiparesis and died 15 days post-SAH. Gross and microscopic examination of this cat revealed severe orbital infection, along with meningitis. Therefore, the cat was eliminated from the study.

Of the 12 cats exposed to SAH in Group III, nine survived for 16 days (75%). The remaining three cats died at 1, 6, and 15 days post-SAH. The only cat in this group that developed hemiparesis was the one that died 15 days post-SAH and was omitted from the study.

**Pathological Alterations**

In Group I (three control animals), vessels appeared normal and consisted of a single layer of endothelial cells, an uncorrugated internal elastic membrane, several layers of smooth-muscle cells, and an adventitia (Fig. 1). In Group II (10 untreated-SAH cats) the right MCA’s distal to the site of rupture showed Grade II pathology. The arteries demonstrated intimal smooth-muscle proliferation three to eight cell layers thick (Fig. 2). The internal elastic membrane was corrugated, and there was myofibrosis (Fig. 2 right). At the site of rupture (not illustrated), arteries showed Grade III arteriopathy. There was a combination of thrombosis and severe intimal proliferation, resulting in almost total occlusion of the vessel lumen. The intimal proliferative
areas contained several vascularization channels and many large myofibroblast-like cells. Corrugation and disruption of the internal elastic membrane, along with severe shrinkage and fibrosis of the media of the vessel, could also be demonstrated. The left MCA's, contralateral to the side of rupture, showed Grade I pathology consisting of mild intimal proliferation of both endothelial cells and smooth-muscle cells, mild splitting and corrugation of the internal elastic membrane, and occasionally myonecrosis.

In Group III (nine cats with heparin treatment and SAH), the right MCA's distal to the site of rupture showed Grade I pathology (compared to Grade II in untreated-SAH Group II cats). The most consistent alteration was mild intimal proliferation of two to three cell layers (Fig. 3 left and center). The arteries showed corrugation of the internal elastic membrane, some of the same severity as observed in Group II (Fig. 3 right). The media of the arteries was rarely involved. At the site of rupture (not illustrated), the right MCA's showed Grade II pathology consisting of intimal proliferation of three to eight cell layers, corrugation of the internal elastic membrane, and myofibrosis (compared to Grade III in untreated-SAH Group II cats). However, unlike
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Fig. 4. Diagram of the middle cerebral artery (MCA) showing the main trunk, the branching segment, the point of rupture (arrowhead), and the surgical exposure for suture-hook placement (circle). ACA = anterior cerebral artery; ICA = internal carotid artery.

that observed in Group II, there was no obvious thrombus at the rupture site. The left MCA's (contralateral to the rupture) demonstrated no changes or, occasionally, mild corrugation of the internal elastic membrane. No changes were observed in the basilar arteries of either experimental group.

Cerebral Pathology

Gross and microscopic study revealed infarction of the cerebral cortex in the area of distribution of the right MCA in two Group II cats. These two animals developed left hemiparesis in their post-SAH course. Infarction was most pronounced along the course of the main trunk of the vessels, and correlated well with anatomical alterations within the vessel wall. There was no evidence of cerebral infarction in Group III animals.

Propagation of Vessel Pathology

Figure 4 is a diagrammatic illustration of various arterial segments of the circle of Willis, the region of surgical exposure of the artery, and the site of suture-hook placement in the wall of the right MCA. In the cat, the MCA consists of a "main trunk" approximately 8-mm long before significant primary and secondary branching occurs. Distal to the 8 mm trunk, branching is very frequent and arterial segments become smaller. Approximately 12 mm of the "branching segments" of the artery could be successfully removed for study. We used the previously described surgical approach, and placed the suture-hook more proximal than distal in the main trunk of the vessel (Fig. 4).

Figure 5 represents a reconstruction of the propagation of subintimal proliferation in the right MCA 16 days following SAH in untreated cats (Group II). At the site of rupture, proliferation was very severe, resulting in almost total occlusion of the lumen. Such proliferation was continuous for approximately 1.5 mm, spreading an equal distance proximal and distal to the site of rupture. Proximal to the rupture site, continuous proliferation became milder and discontinuous for approximately 2 mm. Distal to the site of rupture, continuous proliferation also became milder and discontinuous at approximately 4 mm. Therefore, the entire length of the main trunk of the right MCA showed some degree of vessel damage. When the branching segment of the same vessel was examined, mild discontinuous proliferative subintimal changes were observed throughout.

Fig. 5. Diagrams showing the propagation of subintimal proliferation. Density of geometric shapes located along the intimal layer of the vessel indicates the presence and intensity of intimal proliferation. The arrow indicates the point of vessel rupture in the right middle cerebral artery (MCA) (A and C); the contralateral MCA's are also shown (B and D). A and B: Group II arteries from untreated cats. C and D: Group III arteries from heparin-treated cats.
its full extent. Figure 5B represents the propagation of proliferation in the left MCA (contralateral to the ruptured side). In the main trunk, proliferation was mild, spotty, and discontinuous. Such changes were seen at intervals along the longitudinal aspect of the vessel for approximately 4 mm of the 8-mm segment. The proliferation seemed to occur more frequently in distal aspects of the trunk than in proximal areas. When the branching segment of the left MCA was studied, mild focal proliferation, very similar to that observed in the right MCA, could be demonstrated throughout the entire 12-mm segment (Fig. 5B).

Figure 5C and D represents the spread of subintimal proliferation 16 days post-SAH in the MCA's of cats treated with heparin. At the site of rupture (right MCA), smooth-muscle proliferation was continuous for approximately 0.8 mm. Distal to the point of rupture, mild discontinuous proliferation skipped along the main trunk and into the branching segment (Fig. 5C). The frequency and severity of proliferation along the longitudinal aspect of the artery was less than in its untreated counterpart (Fig. 5A). Proximal to the point of rupture, no proliferation could be demonstrated. The left MCA showed no proliferation (Fig. 5D).

Discussion

Studies from this laboratory have documented the pathological changes in the major cerebral arteries following a closed-space SAH due to vessel laceration in the cat. In that model, proliferation of smooth-muscle cells and fibroblasts on the luminal side of the intimal surface of the artery has been documented at 16 days after arterial rupture. We have proposed that this proliferative angiopathy is stimulated by a growth factor or factors released from platelets that adhere to the intimal surface of the artery.

At least one such factor, platelet-derived growth factor (PDGF), has been shown to stimulate proliferation of fibroblasts and arterial smooth-muscle cells in culture. This factor is not active in heparinized plasma. Guyton, et al., have reported that heparin administration dramatically reduces the myointimal thickening that follows arterial injury in rats. They have suggested that the effect of heparin on the injured arterial wall is primarily to inhibit smooth-muscle cell growth, and that this effect is not related to anticoagulant activity. Hoover, et al., have subsequently confirmed that heparin inhibits growth of both smooth-muscle cells and fibroblasts in cell culture, and that its effect is due not only to competitive inhibition of growth factors in solution but also to its binding to cell surfaces and blocking access to receptors for the platelet factors. Recently, Grotendorst, et al., have shown that PDGF, but not other mitogens, is chemotactic for smooth-muscle cells. Since heparin is an inhibitor of PDGF, its administration to patients following SAH may prevent the development of proliferative angiopathy which may cause structural stenosis, as well as loss of compliance of the larger cerebral arteries. There appears to be general agreement that heparin does not inhibit endothelial cell growth, and thus would not delay ultimate healing of the arterial lesion. Hoover, et al., found that the addition of heparin to cell cultures significantly stimulated endothelial cell growth, although Guyton, et al., and Clowes and Karsnows' revealed that heparin did not accelerate the rate of endothelial cell growth.

In a recent review of the extensive literature on the effects of platelet-inhibiting drugs in endothelial injury models of atherosclerosis, Saunders noted that aspirin, dipyridamole, prostacyclin, papaverine, isoproterenol, nifedipine, and nitroglycerin did not inhibit the mitogenic effect of PDGF in cell cultures. Following endothelial injury, sulfipyrazone reduced in vivo smooth-muscle cell proliferation in rabbit iliac artery but not in guinea pig aorta; however, dipyridamole, aspirin, flurbiprofen, and resperine had no effect. Drugs other than heparin which have been reported to inhibit PDGF are fenofibrate and trapidil.

The debate as to whether cerebral ischemia following SAH is due to contraction of vascular smooth-muscle cells or to structural changes in the arterial wall has not been resolved. The arterial changes seen in this laboratory were originally described or have been substantiated by Conway and McDonald, Mizukami, et al., Peerless, et al., Crompton, and Hughes and Schianchi. Others have found morphological changes in cerebral arteries, which they have ascribed to the presence of spasm. There are many possible explanations for these conflicting reports, including the experimental model chosen, whether a vessel is torn or blood is injected into the subarachnoid space, the animal species, time of examination, and method of examination. The latter appears to be especially critical since some of the most important changes are best seen by a very specific method of examination; for example, intimal surface changes of an artery are best appreciated when the artery is examined by scanning electron microscopy. Blaumanis found marked endothelial damage, an intimal platelet carpet, and thrombi after periarterial application of blood to the basilar artery. Alksne and Branson also found compatible endothelial changes after periarterial hemorrhage. Mayberg, et al., failed to demonstrate similar endothelial changes following intracisternal blood injections; however, they suggested that the ischemic deficit attributed to vasoconstriction is not thromboembolic in etiology, since the evolution of neurological deficits is much slower when compared to those seen after embolic conditions.

This study demonstrates a definite and unequivocal absence of proliferative angiopathy in arteries remote from the rupture site in the heparin-treated animals, whereas proliferative angiopathy was present in 100% of untreated-SAH animals. Further, proliferative angiopathy was dramatically reduced at or near the arterial rupture site in animals treated with heparin when compared to non-treated cats. These changes would be
Heparin in post-SAH proliferative angiopathy expected if our hypothesis is correct that PDGF, which is released from the platelet carpet upon the intimal surface of the vessel, is the stimulus for development of proliferative angiopathy.

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References


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