Cerebral arterial pathology in experimental subarachnoid hemorrhage

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Pathological changes of the cerebral arteries were studied in 30 dogs after subarachnoid injections of saline, fresh autologous blood, epinephrine, blood plus epinephrine, norepinephrine, or blood plus norepinephrine. Macroscopically, the circle of Willis was maximally dilated after the injection of epinephrine and was constricted following administration of blood plus epinephrine. Microscopically, neither saline nor blood produced abnormalities, except for minor changes of the adventitia in the latter. Epinephrine produced frank necrosis of smooth-muscle cells, which was subsequently replaced by fibrosis in the media of larger subarachnoid arteries, and the leakage of necrotic material from the infarcted hypothalamus contributed to these lesions. Blood plus epinephrine produced marked changes in the internal elastic lamina and tortuosities of the nuclei of smooth-muscle cells, while norepinephrine and blood plus norepinephrine produced only minor changes.

Previously reported findings of morphological changes due to vasospasm after subarachnoid hemorrhage were confirmed experimentally, but such changes were found only after application of epinephrine. It is suggested that epinephrine produced the most severe vasospasm among the five substances tested.

Key Words - cerebral artery • experimental subarachnoid hemorrhage • vasospasm • myonecrosis • light microscopy

Cerebral vasospasm following subarachnoid hemorrhage (SAH) is a major unsolved problem in the treatment of cerebral aneurysms. There is a higher incidence of vasospasm with aneurysms of the anterior portion of the circle of Willis. The sympathetic nervous system was implicated in the etiology of vasospasm by such observations as refractory hypertension, arrhythmias, electrocardiographic abnormalities, and myocardial damage.17 Peerless and Griffiths15 demonstrated significantly elevated plasma levels of norepinephrine and epinephrine, usually 5 to 10 days after SAH.

Vasospasm causes severe ischemia in the territories of the affected arteries without histological evidence of occlusion. Structural changes ascribed to vasospasm have been studied not only in human autopsy material but also in numerous animal models. Since the first report of Crompton,4 several authors1-2,7,10-12,14,16 have demonstrated that vasospasm may cause morphological changes in the involved segments of cerebral arteries. They suggested that vasospasm is not merely due to the contraction of smooth-muscle cells, but to various intimal changes corresponding to the degree and duration of the spasm. However, the microscopic changes in cerebral arteries following vasospasm could not be clearly distinguished from ordinary atherosclerotic or secondary hypoxic changes developing distal to the spastic arteries, or from artifacts following operative procedures. Peerless, et al.,14 demonstrated differences in the cerebral arterial lesions of human and experimental SAH.

Although Alksne and Greenhoot,1 Fein, et al.,7 and Tani, et al.,16 produced myonecrotic changes by subarachnoid injection of blood or norepinephrine, such changes were limited to a small number of smooth-muscle cells, and were observed only by electron microscopy. There are no systematic studies of the cerebral arterial pathology in experimental SAH that show positive microscopic findings.

We have attempted to study by light microscopy the structural changes that occur in the walls of the cerebral arteries following experimental introduction of blood and/or catecholamines into the canine subarachnoid space.

Materials and Methods

Adult dogs, each weighing 8 to 13 kg, were anesthetized with intravenous administration of Nembutal (pentobarbital). The test materials were injected into
the chiasmatic cistern through the optic canal, using a spinal catheter 0.80 × 70 mm in size. All animals with the slightest evidence of artificial bleeding from the spinal catheter were excluded from the experiment. Approximately 0.4 ml/kg of cerebrospinal fluid (CSF) was removed by spontaneous dripping from the cannula before injection. The CSF was examined microscopically to verify the absence of red blood cells. In order to inflict greater damage to the circle of Willis and to simulate the conditions prevailing in human SAH, the dogs were subjected to rapid manual injection of the test materials. Respiratory arrest often occurred immediately after the injection of all agents except saline, but the dogs usually tolerated the procedure upon artificial ventilation for 5 to 30 minutes. The material injected was 1.0 ml/kg body weight of saline (control group), 0.8 ml/kg of fresh autologous blood, 0.15 mg/kg of epinephrine, 0.65 ml/kg of blood plus 0.15 mg/kg of epinephrine, 0.15 mg/kg of norepinephrine, or 0.65 ml/kg of blood plus 0.15 mg/kg of norepinephrine.

For each test material, more than 10 dogs were used to obtain five experiments without artifacts. The results reported here pertain to 30 dogs, five for each of the six different test materials. Angiography was never performed for fear of causing artifacts in the intima. Animals were sacrificed 3 days, 7 days, 10 days, 3 weeks, and 4 or 6 weeks after the experiment by exsanguination from the femoral artery. The brain was removed immediately through a wide opening of the skull, and was placed in 10% formalin overnight. The distribution of the blood or fibrinous clot and the
FIG. 2. Photomicrographs from dogs after subarachnoid epinephrine injections. **Upper Left:** Internal carotid artery, 1 week after the experiment. There is almost complete necrosis of smooth-muscle cells, and a dilated lumen. Numerous red blood cells as well as inflammatory cells are scattered through the subarachnoid space. The adjacent hypothalamus shows hemorrhagic infarction, with an accumulation of numerous red blood cells, foamy granular cells, blood proteins, and astroglial proliferations. H & E, × 61. **Upper Center:** Internal carotid artery, 1 week after the experiment. The media is thin and fibrotic, resulting from extensive necrosis of smooth-muscle cells. The subarachnoid space is filled with granulation tissue. Azan-Mallory, × 61. **Upper Right:** Internal carotid artery, 3 weeks after the experiment, showing marked dilation with myonecrotic changes. The adventitia can hardly be differentiated from the fibrous tissue, which completely obliterates the subarachnoid space. H & E, × 61. **Lower:** Small subarachnoid arteries, 1 week after the experiment. A mixed population of red blood cells, fibrinoid substances, and inflammatory cells are seen within the swollen wall of the arteries. H & E, × 125.

Results

Saline

Injection of saline did not produce any gross or microscopic changes in the subarachnoid vessels. The subarachnoid space was verified to be free of artificial bleeding.

Fresh Blood

The injection of fresh blood produced a remarkable inflammatory reaction in the subarachnoid space (Fig. 1). Large numbers of inflammatory cells and degenerating red blood cells were seen scattered among the increased numbers of collagen fibers and trabeculations in the subarachnoid space. The arterial walls were almost normal except for the adventitia. The latter showed slight thickening, presumably due to the surrounding hemorrhage. The internal elastic lamina often showed increased tortuosity. Resorption of the blood was nearly complete 10 days after the experiment, leaving no visible remnants of the clot except in the prepontine cistern.

Epinephrine

Although the injection of epinephrine was performed completely without artificial bleeding, pathological examination (Fig. 2) surprisingly demonstrated
evidence of SAH and fibrosis, even 2 weeks after the experiment. This was in remarkable contrast to the injection of blood, which, by this time, had been almost completely resorbed.

Macroscopically, there was also striking vascular dilatation. Injection of epinephrine produced remarkable microscopic changes, not only in the vessels but also in the subarachnoid space and in the adjacent brain tissue. One week after the experiment, the subarachnoid space was packed with delicate trabeculations of fibrous tissue, interspersed with erythrocytes, plasma cells, and macrophages laden with red cells. Fibrosis of the arachnoid appeared around the circle of Willis, and became increasingly evident as the weeks advanced. Between 2 and 3 weeks, fibrosis was seen to fuse the pia and arachnoid, obliterating the subarachnoid space, particularly that adjacent to the hypothalamus. By that time, red blood cells and inflammatory cells had decreased.

Pathological changes of the cerebral vessels were seen both in the circle of Willis and in the small subarachnoid arteries. The circle of Willis showed a marked dilatation of the arterial lumen, and a frank necrosis of the media. More than half to almost the entire layer of smooth-muscle cells was necrotic and replaced by fibrosis. Myonecrosis of the larger subarachnoid arteries was observed in three of the five dogs, which were sacrificed 1 week, 10 days, and 3 weeks after the experiment. All of them had additional hypothalamic lesions. The internal elastic lamina became generally flattened. The adventitia either adhered to increased numbers of fibroblasts and collagen fibers, or was embedded within a completed fibrous tissue. The small subarachnoid arteries had a swollen
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FIG. 4. Photomicrographs from dogs after subarachnoid norepinephrine injections. Left: Posterior communicating artery, 10 days after the experiment. The endothelial cells are lifted to form a subintimal space containing inflammatory cells, erythrocytes, and an exudate of blood protein. H & E, × 125. Right: Hypothalamus, 10 days after the experiment, showing an ischemic infarction with foamy granular corpuscles. The subarachnoid space is filled with increased collagen fibers. The small subarachnoid arteries have a thickened vascular wall, containing erythrocytes and dark particles. H & E, × 62.

Blood Plus Epinephrine

Gross inspection of brains of dogs treated with blood plus epinephrine showed a slight remnant of SAH even 3 weeks after the experiment, especially in the optic chiasm and the interpeduncular or prepon- tine cistern (Fig. 3). Resorption of the blood evidently took much more time than after the injection of fresh blood. The most striking macroscopic finding was that the larger subarachnoid arteries generally showed severe narrowing, in sharp contrast to the dilated arteries following epinephrine. A small ischemic infarction of the hypothalamus was observed in only one of the five dogs in this group. Microscopically, the injection of blood plus epinephrine produced a moderate degree of inflammatory reaction in the subarachnoid space as well as severe vasoconstriction. A moderate number of inflammatory cells (such as lymphocytes, plasma cells, and macrophages laden with hemosiderin) were seen in the subarachnoid space. Perivascular cuffing of such inflammatory cells was predominant, especially around the subarachnoid veins. Degenerating red blood cells decreased in number until 3 weeks after the experiment, while the quantity of hemosiderin, pigment-laden macrophages, and delicate strands of fibrous tissue increased. The media of the subarachnoid arteries was usually thick and had a slight foamy degeneration of smooth-muscle cells. The nuclei of smooth-muscle cells were generally small and turgid, suggesting long-standing severe vasospasm. The internal elastic lamina was most abnormal, being unusually infolded, corrugated, split, and disrupted. On dissection, the internal elastic lamina occasionally formed intramural spaces (containing red blood cells and blood proteins) communicating with the arterial lumen.

Norepinephrine

Gross inspection of the brains of dogs in the nor- epinephrine group showed slight fibrosis and thick- ening of the arachnoid 10 days after the experiment (Fig. 4). Eventually, the subarachnoid space adjacent to the hypothalamus was completely obliterated by fibrosis. The larger subarachnoid arteries showed a slight constriction.

Microscopically, the injection of norepinephrine did not produce any histological changes in the larger arteries, but alterations were seen in the small subarachnoid arteries and in the subarachnoid space. The subarachnoid space adjacent to the hypothalamus was filled with increased collagen fibers and fibrin, numerous polymorphonuclear leukocytes, lymphocytes, macrophages laden with hemosiderin, and only sparse degenerating red blood cells. The majority of the larger subarachnoid arteries revealed no abnormalities, except for one showing dissection of the endothe- lium with a subintimal space containing many inflammatory cells. The small subarachnoid arteries, especially those embedded in fibrosis, were thickened from...
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Fig. 5. Photomicrographs from dogs after subarachnoid injections of blood plus norepinephrine. H & E, × 62. Left: Middle cerebral artery, 2 weeks after the experiment. The smooth-muscle cells of the media show a moderate degree of vacuolar disruption. Right: Internal carotid artery, 6 weeks after the experiment. Some of the smooth-muscle cells are tortuous, and the internal elastic lamina is markedly corrugated and partially detached, infolding into the arterial lumen.

increased connective tissue, scattered intramural red blood cells, and dark particles, suggesting increased permeability. Myonecrosis of the larger subarachnoid arteries was never observed. One of the five dogs had a small ischemic infarct of the hypothalamus.

Blood Plus Norepinephrine

Gross inspection of the brains of dogs treated with blood plus norepinephrine showed a slight remnant of SAH and thickening of the arachnoid 2 weeks after the experiment (Fig. 5), but no residue of SAH at 3 weeks. Resorption of the blood took place faster than after the injection of blood plus epinephrine, but longer than after that of blood alone. The larger subarachnoid arteries showed moderate constriction.

Microscopically, the injection of blood plus norepinephrine produced inflammatory reaction in the subarachnoid space, but there were only minor changes of the arterial wall. There were moderate numbers of inflammatory cells (mixed with degenerating red blood cells, hemosiderin or hematoidin pigment, and blood substances) scattered among an increased number of collagen fibers. Even 6 weeks after the experiment, the larger subarachnoid arteries generally revealed a moderate degree of vasoconstriction with vacuolar disruption in the media and corrugation of the internal elastic lamina.

Discussion

Myonecrosis

The present experiments demonstrated visible narrowing of the larger subarachnoid arteries, over a period of more than 3 weeks in the group that had received an injection of blood plus epinephrine, and over a period of 6 weeks in the group that had received an injection of blood plus norepinephrine. The introduction of epinephrine, however, produced striking vasodilatation, which microscopically corresponded to frank myonecrosis. This was consistent with the findings of Mizukami, et al., who reported that most arteries examined more than 2 weeks after clinical vasospasm showed dilatation of the arterial lumen and frank necrosis of the smooth-muscle cells. In our study, epinephrine induced the most severe vasospasm among the five materials tested.

Alksne and Greenhoot observed on electron microscopy slight degrees of myonecrosis of the basilar artery following subarachnoid injections of norepinephrine in monkeys. Tani, et al., produced vaso-spasm in canine basilar arteries after the injection of fresh arterial blood or norepinephrine; they also found that myonecrosis was limited to a small number of the smooth-muscle cells. Recently, Eldevik, et al., stated that artificial SAH produced by the injection of autogenous blood into the cisterna magna in dogs gave rise to considerable narrowing or spasm of the basilar artery; however, none of the animals showed abnormalities in the intima or the media of the vessel walls. No one has ever produced such a distinct myonecrosis, so clearly confirmed microscopically, as in the present study.

The myonecrosis produced by epinephrine in our experiments was similar to the data shown in Fig. 13-1 of Peerless, et al., and in Figs. 6-b and 14-15 of Mizukami, et al., in human autopsy material. The fact that myonecrosis predominated in the outer layer of the media suggests that vasoactive substances affect the arterial wall not from within the arterial lumen, but from outside.

Role of Hypothalamic Lesions

Peerless and Griffiths demonstrated significantly elevated levels of plasma norepinephrine and epinephrine, usually 5 to 10 days after SAH. As the
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catecholamine concentration in the circulating blood may be too low to explain the prolonged vasospasm, it is possible that other vasoactive substances may have contributed as well. There is evidence suggesting that blood in the subarachnoid space is not the only factor involved in vasospasm; hypothalamic dysfunction may also be important for its development. The present observations, that three animals with myonecrosis of the larger subarachnoid arteries always had associated hypothalamic lesions, may suggest a close relationship between hypothalamic lesions and the occurrence of vasospasm. Crompton and Doshi and Neil-Dwyer demonstrated numerous hypothalamic lesions in patients who had died after SAH. It is possible that injuries to the hypothalamus may result in the liberation of vasoactive agents including epinephrine into the subarachnoid space, bathing the larger subarachnoid arteries. Conversely, hypothalamic infarction may be the consequence of excessive vasospasm. A possible explanation of the fact that only epinephrine induced such severe myonecrosis is that this agent might have predominantly damaged the small subarachnoid arteries supplying the hypothalamus.

Blood-CSF Barrier Disturbance

Fox and Ko and Mizukami, et al., described an apparent leakage of contrast material in the region of the circle of Willis on enhanced computerized tomography (CT) performed within several days after SAH. Pi and other authors also stated that SAH often causes a disturbance of the blood-CSF barrier. All of these authors suggested that the apparent leakage of contrast medium might occur through the parent vessels and/or vasa vasorum, and precede the onset of vasospasm. Although the present experimental results were consistent with their findings, we believe that the leakage of blood substances occurred not from the parent vessels or vasa vasorum, but mainly through the interstices of the small subarachnoid arteries. A mixed population of red blood cells, fibrinoid materials, and inflammatory cells, which were observed within the swollen wall of the small subarachnoid arteries, suggested their increased permeability.

Hammes reported that the intensity of the subarachnoid reactions was at its peak 7 days after human SAH. It is interesting that evidence of contrast medium leakage seen on CT was present only in the early stage of SAH, and was no longer visible during the phase of actual vasospasm. These two phenomena are probably due to the fact that, once vasospasm has set in, the interstices of the small subarachnoid arteries may close up, rendering contrast medium leakage insignificant.

Intimal Pathology

The present observations disclosed an uncommon lesion of the endothelial cells in all but one of the dogs injected with norepinephrine. The subintimal accumulation of inflammatory cells observed in that animal was similar to the finding of Crompton, who demonstrated a marked subendothelial leukocytic infiltration in human autopsy material. However, the histological changes of the internal elastic lamina were of such a degree that the arterial lumen became narrower, especially following the introduction of blood plus epinephrine or blood plus norepinephrine. Detailed examinations of contracted arteries in human autopsy material demonstrated a progressive accumulation of a cellular-fibrinous material within the intima. In the present experiment, we failed to reproduce such changes except for the subendothelial accumulation of inflammatory cells mentioned above. Peerless, et al., stressed that there is significantly less cerebral arterial pathology, especially in the intima, in experimental SAH than in human SAH. The vascular narrowing due to the subendothelial fibrous reaction noted by Conway and McDonald or Hughes and Schianchi was related to the fact that it occurs much later than clinical vasospasm. It is one of the purposes of this report to suggest that the subendothelial fibrous reaction noted by these authors should be more carefully differentiated from atherosclerosis, artifacts from operative procedures, or secondary ischemic changes due to vasospasm itself. It is suggested from the present study that organic luminal narrowing is not due to intimal pathology of subendothelial fibrous reaction, but rather to intimal pathology of subendothelial edema or inflammatory cellular reaction, and to the pathological changes of the internal elastic lamina, such as infoldings, detachment of the internal elastic lamina, and subendothelial accumulation of blood substances.

Conclusions

The present experiments have led us to the conclusion that vasospasm is due to long-standing contraction of smooth-muscle cells, induced by epinephrine and exudates from the small subarachnoid arteries or the hypothalamic lesions, as well as to stenosis of the arterial lumen by the pathological changes of the internal elastic lamina and subendothelial accumulations of blood substances.

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References


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