Sequential changes of vascular ultrastructure in experimental cerebral vasospasm

Myonecrosis of subarachnoid arteries

Divisions of Neurosciences and Physiology, Armed Forces Radiobiology Research Institute, Bethesda, Maryland, and Department of Neurosurgery, Georgetown University School of Medicine, Washington, D.C.

The authors describe the striking ultrastructural evolution of vacuolar degeneration of the media in subarachnoid arteries that have been in spasm following subarachnoid hemorrhage.

KEY WORDS ultrastructure cerebral vasospasm subarachnoid hemorrhage

Theoretical and clinical observations support the notion that cerebral vasospasm occurs in a biphasic fashion related to the particular species and exciting causes. Severe vasoconstriction is clinically noted shortly after spontaneous subarachnoid hemorrhage (SAH) and in experimental animals may be consistently reproduced by mechanical, chemical, or electrical stimulation. The role of the sympathetic nervous system in acute experimental spasm is suggested by the rich innervation of cerebral arteries, the depletion of catecholamines from nerve endings after subarachnoid injection of blood, and the resolution of acute spasm in cats by topical and parenteral alpha adrenergic blockade. Thus, a growing consensus views cerebral vasospasm in terms of a tonic contractile mechanism probably under sympathetic influence. Current trials of adrenergic blocking agents as well as sympathectomy, however, yield conflicting results when monitored clinically, radiographically, or by cerebral blood flow measurement.

It is possible that after spasm is triggered by sympathetic discharge, irreversible changes may occur in the vasculature so that sympatholytic agents alone no longer cause spasmolysis. Previous studies of autoregulation of blood flow in rhesus monkeys after SAH suggest a loss of vasoelasticity. Whether the luminal narrowing, most commonly seen angiographically in arteries greater than 250 μ diameter, is due to a tonic energy consuming contractile process alone or simply due to reactive changes in
the arterial wall is unknown at present. The significant role of these subarachnoid arter-
ies in modulating cerebral blood flow, however, is reflected in the finding that
relative pressure drops in arteries upstream from the large surface arterioles (> 300 μ)
vary between 33% and 39% depending on systemic blood pressure.31

In this series of animals a morphological approach was used to study changes in these
arteries (> 240 μ) after angiographically demonstrable vasospasm.

Methods

The data from 18 rhesus monkeys were considered reliable and free of artifact. Blood pressure and arterial blood gases were monitored through a polyethylene

---

**FIG. 1.** Duration and severity of vasospasm in 18 monkeys. Induced SAH resulted in angiographically demonstrable vasospasm in 14 animals.

**FIG. 2.** Electron micrograph of endothelial surface of normal cerebral artery showing a single layer of spindle-shaped endothelial cells (E) lining the lumen (L). These cells are joined by tight junctions (arrows), often with overlapping cytoplasmic flaps, and contain pinocytotic vesicles, mitochondria, and occasional lysosome-like bodies, and cytoplasmic filaments. Elastic lamina (el) separates endothelium from the smooth muscle cell layer (M). Occasional "insertions" (asterisk) of muscle cell processes are observed through the elastica attaching to the endothelial cells. Collagen (c). × 18,000.
catheter inserted transfemorally into the abdominal aorta and maintained chronically with a Luer-Lock cap after heparinization. Arterial blood gases were obtained daily, and animals with a pO₂ lower than 65 mm Hg were discarded from the study, to insure that morphological changes were not due to generalized hypoxemia. In 11 monkeys intracisternal injection of 3 cc of fresh isologous arterial blood was utilized to induce spasm, while in seven monkeys a frontotemporal craniectomy was performed and the intradural internal carotid artery was punctured with a No. 30 gauge needle. At 20 minutes to 1 hour after SAH, angiography was performed via the right transbrachial route and repeated at intervals of 1 or 2 days until sacrifice.

Diameters of the anterior, middle, and posterior cerebral arteries were measured on the radiographs at 4 mm intervals beginning at their origins. The sum of these diameters was recorded as the Arterial Index (AI). Since a variability of up to 23% was noted in repeat control angiograms of the same animal, a reduction of AI greater than 25% was used to define the presence of vasospasm.

Specimens were fixed for histological and ultrastructural study by vascular perfusion. After thoracotomy, the left ventricle was cannulated, the descending aorta clamped, and blood washed from the head and cervical regions with McEwen’s saline followed by Karnovsky’s formaldehyde-glutaraldehyde fixative one-fourth strength and then repeated full strength. After fixation in situ for at least 3 hours, cerebral arteries were dissected free of the brain parenchyma and washed and stored in cacodylate-buffered 10% sucrose. Segments of interest were further dissected into small blocks, post-fixed in cacodylate-buffered 1% osmium tetroxide, and processed through graded alcohols, uranyl acetate block stain, and propylene oxide for embedding in Epon 812. Thick sections (1.0 to 1.5 μ) were cut on an Ultramicrotome* and stained with a mixture of methylene blue and azure II for light microscopy. Areas for ultrastructural study were selected, and thin sections (600 to 1200 Å) were cut on a diamond knife, stained with uranyl acetate and lead citrate, and examined with an electron microscope.†

Results

Angiographic Spasm

Fourteen (70%) animals developed spasm, and in six (33%) it persisted for longer than 1 week (Fig. 1). Two normal animals as well as animals that did not develop vasospasm after subarachnoid injection of blood (3) or puncture (1) served as controls. Intracisternal injection of fresh arterial blood produced spasm lasting 1 to 3 days while all the cases of chronic spasm (> 7 days) resulted from needle puncture of the intradural internal carotid artery. Spasm after puncture was usually confined to the ipsilateral subarachnoid arteries, but the distribution after cisternal injection was more random.

Ultrastructure of Normal Cerebral Arteries

The subarachnoid arteries were lined by a layer of spindle-shaped endothelium, containing occasional pinocytotic vesicles, mitochondria, and membrane-bound lysosomal-like structures (Fig. 2). The cells were joined by tight junctions showing overlapping or interdigitation. The abluminal aspect of the elastica was of uniformly low electron density, and its luminal aspect was lined with more electron-dense material. The media consisted of smooth muscle cells surrounded by basement membranes and intercellular collagen (Fig. 3). These fusiform cells contained a central “core” of nucleus plus organelles (mitochondria, endoplasmic reticulum, etc.) surrounded by well-organized muscle filament bundles. Vesicles were prominent just beneath the membranes of the smooth muscle cells. Muscle cells closest to the lumen were inserted into fenestrae of the internal elastic laminae. Occasional nerve fiber bundles in the adventitia contained both myelinated and nonmyelinated fibers. Sections of cortical arteries taken from normal animals, and

---

*MT-2 ultramicrotome manufactured by Sorvall Company, Norwalk, Connecticut.
Ultrastructure of Cerebral Vasospasm

Early Spasm (Less than 1 Day). Specimens from this group (two animals) demonstrated a reduction in lumen size with corrugation of the internal elastica which, on the light microscopic level, was indistinguishable from the appearance of normally constricted arteries. On the ultrastructural level, however, early electron lucent changes in the muscle cells were noted within 8 hours, especially at the crests of the corrugations in the elastica. Condensed lysosomes and degenerating mitochondria were present within occasional muscle cells, and lipid figures were observed among the pinocytotic vesicles beneath the sarcolemmal membrane (Fig. 4).

Prolonged Spasm (2 to 7 Days). Specimens (seven animals) demonstrated rounding of the endothelial cell nuclei and cytoplasm (Fig. 5) with cell processes assuming a flattened configuration along the elastica and a prominent loss of tight connections between endothelial cells (Fig. 6). Even after fixation by vascular perfusion, platelets were observed adherent to the altered endothelial surface, both near the rounded endothelial cells and along denuded regions of the elastica itself. The elastica remained dense along its luminal edge, and the most visibly affected regions were most severely corrugated. Smooth muscle cells were more electron dense than normal, and those nearest the adventitia contained intracytoplasmic vacuoles of various sizes and content, some appearing “empty” and others containing a light granular, or denser, more amorphous material (Fig. 7). On the other hand, smooth muscle cells subjacent to the crests of the elastica contained degenerated organelles and lipid figures. Throughout the
Vascular ultrastructure in cerebral vasospasm

Fig. 4. Electron micrograph showing medial changes in early spasm. Occasional muscle cells (M) are observed with condensed lysosomes and degenerating mitochondria (asterisk) within the central core of organelles. Basement membrane (bm). Muscle filaments (f). × 18,000.

media there was a decrease in the number of pinocytotic vesicles below muscle cell membranes. In the most dramatic sections, frankly pyknotic muscle cells were encountered. Occasional changes were observed in the nerve fiber bundles, consisting of degenerating lipid in the cytoplasm of Schwann cells and lipid figures in axis cylinders.

Chronic Vasospasm (More Than 1 Week). The endothelial cells in these arteries (six animals) assumed a more normal spindle-shaped configuration (Fig. 8) with tight intercellular junctions and an increase in cytoplasmic filaments. The entire internal elastic laminae remained somewhat more electron dense than normal. Numerous smooth muscle cells still contained large vacuoles as previously described. Throughout the media were muscle cell remnants of increased electron density (Fig. 9) with loss of complex internal structure, surrounded by increased amounts of intercellular collagen.

Relationship Between Angiographic and Electron Microscopic Vasospasm

The areas of most severe angiographic spasm were identified in early, prolonged, and chronic vasospasm. It was noted that spasm in the first few hours after puncture is very evanescent and variable with little correlation between the location of microscopic and angiographic sites. In prolonged and chronic vasospasm there was more consistency in the location and severity of the maximal spasm and a better correlation with areas of microscopic spasm.
Discussion

Pathological studies of the intracranial arteries following vasospasm are incomplete. Crompton\textsuperscript{6,7} noted frank necrosis of cortical arteries and veins in the vicinity of Sylvian hematomas after rupture of middle cerebral artery aneurysms. Conway and McDonald\textsuperscript{8} described subendothelial granulation in the intradural arteries of 12 patients surviving 4 weeks or more after SAH. Six of these cases had actual luminal narrowing due to subendothelial thickening while the media and adventitia appeared histologically normal.

Despite the clearly abnormal appearance of the larger (diameter greater than 250\(\mu\)) subarachnoid arteries seen at angiography, no systematic effort has been made to study the fine structure of these vessels in vasospasm as a result of the requirement for immediate perfusion fixation. Such studies might demonstrate the locus of pathology within the arterial wall and differentiate changes specific for vasospasm from the secondary effects of anoxia.

It is apparent from the present study that vessels in early spasm are indistinguishable from normally constricting arteries under light microscopy. At the ultrastructural level, changes were noted in the muscularis and internal elastica at 8 hours. In early spasm, however, the structural changes bore little topographic relationship to the angiogram. When spasm remained localized to the same segment for over 1 week, corresponding histological changes were invariably found. Smooth muscle cells were most consistently and severely affected, but there were secondary changes in the intima and nerve fiber bundles. These muscular changes may reflect a cycle of increased mechanical and metabolic work in the presence of decreased cerebral blood flow. This would produce sarcolemmal breakdown, and potassium release to reinforce norepinephrine-induced tonic contraction,\textsuperscript{28} and eventually death of the muscle cell in
Vascular ultrastructure in cerebral vasospasm

Fig. 6. Electron micrograph showing endothelial surface changes in prolonged spasm. There is a prominent loss of tight connections (arrows) between the endothelial cells formerly completely covering internal elastic lamina (el). Numerous platelets (P) are observed adherent to the denuded elastica and remaining flattened endothelial processes, in spite of fixation by vascular perfusion. Lumen (L). × 18,000.

diastole. In this connection Alksne and Greenhoot² have recently demonstrated myonecrosis of vascular smooth muscle after subarachnoid injection of norepinephrine in the rhesus monkey. We doubt that the preponderance of changes in the media is a peculiarity of the experimental animal but may be found if carefully looked for in human material. The subendothelial fibrous reaction noted by Conway and McDonald⁵ may be related to the much longer time course in the latter studies.

Platelet-like bodies were found adherent to endothelial cells and insinuated into the open “junctions” between cells. These are reminiscent of the small granular aggregates noted after traumatic spasm by Symon³² and are probably nonspecific indicators of vascular injury rather than of etiological importance.

The significance of vasospasm is questionable in any particular case, although it is generally considered to be an unfavorable sign, placing the patient with SAH at greater risk.¹ Abnormalities of cerebral blood flow have been noted in clinical cases.³ A biphasic decrease in flow was noted by Simeone, et al.,³⁰ while Fein and Boulous¹² found a biphasic disturbance of autoregulation in rhesus monkeys. In early spasm, autoregulation was impaired during hypertensive stimuli, while in the presence of infarction it was lost to hypotensive stimuli. Whether the disturbance in flow is at all related to the “spasm” visible in the larger arteries has been questioned by some,¹⁶ and the microcirculatory disturbances engendered have been largely unexplored. As a first step it seemed important to identify what, if any, structural aberrations occur in vessels with angiographically demonstrable pathology. The presumed changes in elasticity secondary to the morphological changes found may partially explain the loss of
Fig. 7. Electron micrograph showing changes in prolonged spasm. Adjacent smooth muscle cells (M) are seen, one of normal appearance and one containing large vacuoles (v) with light granular contents. × 9350.

vasoreactivity to hypotensive stimuli found in prolonged vasospasm; however, they do not account for the autoregulatory loss during early spasm. Further studies of the microcirculation are warranted and may elucidate the role of the perforating arteries, and the responses of the capillary network to the metabolic and hemodynamic alterations in cerebral vasospasm.

References

2. Alksne JD, Greenhoot JH: Myonecrosis in catecholamine produced chronic vasospasm. Presented at the American Association of Neurological Surgeons, Annual Meeting, Los Angeles, California, April 1973
Vascular ultrastructure in cerebral vasospasm

FIG. 8. Electron micrograph of the endothelial surface in chronic spasm. Endothelial cells (E) resume a more normal spindle-shaped configuration, with tight junctions (arrows) between cells, and with an increase in cytoplasmic filaments (f). The elastic lamina (el) remains somewhat more electron-dense along its luminal side, and muscle cells and muscle cell remnants of increased electron density (M) are observed. Lumen (L). × 14,000.

22. Lieberman AN, Gardner AL, Goodgold AL, et al: Chronic vasospasm following subarachnoid hemorrhage: treatment with phenoxybenzamine. Presented at the combined meeting of the NE and NY Neurological Societies, New York, Mt Sinai Hospital, November 13, 1970
26. Peerless SJ, Yasargil MG: Adrenergic inner-
Fig. 9. Electron micrograph showing medial changes in chronic spasm. The pyknotic muscle cell remnant (M) has increased electron density and loss of complex internal structure. Basement membrane (bm). Intercellular collagen (c). X 20,600.

...vulation of the cerebral blood vessels in the rabbit. J Neurosurg 35:148–154, 1971

This work was presented in part at the Annual Meeting of the American Association of Neurological Surgeons, Los Angeles, California, April 8–11, 1973.

Address reprint requests to: Jack M. Fein, M.D., Department of Neurological Surgery, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461.