Constrictive Endarteropathy Following Experimental Subarachnoid Hemorrhage

Ben R. Clower, Ph.D., R. R. Smith, M.D., J. L. Haining, Ph.D., and J. Lockard, Ph.D.

SUMMARY Rhesus monkeys were subjected to subarachnoid hemorrhage by either the introduction of blood into the subarachnoid space or by mechanically rupturing the middle cerebral artery. Local cerebral blood flow, vascular compliance studies, and histological studies (light and electron microscope) were made of the vessels of all animals. In animals hemorrhaged via vascular rupture, subintimal swelling and thickening was minimally present by 3 days, becoming progressively more severe by one month. Such changes consisted of severe subintimal proliferation, fibrosis of the medial smooth muscle layer and interruption of the internal elastic membrane. Cells in the subintimal proliferative areas examined by electronmicroscopy were seen to have ultrastructural characteristics of smooth muscle. In the subarachnoid-injected hemorrhaged animals, there was no evidence of intimal proliferation or other vascular changes, which was also true of all control vessels. There was a decrease in wall elasticity of vessels exposed to mechanical rupture when compared to those exposed only to injected subarachnoid blood or normal vessels. Local cerebral blood flow was affected very little following the 2 methods of subarachnoid hemorrhage.

Materials and Methods

Production of SAH

Following the determination of control levels of cerebral blood flow in several sites (table), 10 adult rhesus monkeys were subjected to SAH by one of 2 methods. In Group I (5 animals), following anesthesia, 3 ml of autogenous non-heparinized blood was obtained from the femoral vein and injected into the subarachnoid space via the optic foramen. In Group II (5 animals), SAH was produced by arterial rupture using the following technique. After intraorbital exenteration, a small craniotomy was performed adjacent to the optic foramen exposing the middle cerebral artery proximal to the lateral fissure. A 7-0 suture was placed through the wall of the vessel and tied in position. The dura mater was tacked along within the orbit and 7 days were allowed for healing. At the end of this 7 day interval, the ligature was pulled from the vessel's wall resulting in a "closed space" SAH. Following both methods of hemorrhage, daily cerebral blood flow measurements were made from multiple sites (see table) until the animals were sacrificed at 3, 6, 10, 16 and 30 days.

Cerebral Blood Flow Studies

Local cerebral blood flow (LCBF) was measured at several sites (see table) by a modification of the hydrogen clearance technique of Aukland, Bower and Berliner.7 The modification consists of substitution of a stainless steel jeweler's screw affixed to the skull as the reference electrode instead of a calomel electrode. This permits application of the technique to fully conscious animals via chronic implants.6 Electrodes were aseptically implanted using a stereotaxic device.

Beginning 1-3 days following implantation of electrodes, the animals from both groups were placed in a primate chair 5 days per week and simultaneous measurements of LCBFs at up to 6 sites made over a period of 4 weeks before hemorrhaging. The animal's head must be covered with a plexiglass dome or head...
chamber so as to control the atmosphere breathed. Portholes permit the introduction of gas mixture, exhaust and placement of an O₂ electrode.

For each determination, each platinized electrode was electrically balanced against a corresponding reference electrode by means of the 1,000 ohm potentiometer in the circuit. This null point was set up scale ("zero") in order to facilitate determination of whether the recorder pen returned to recorder "zero" following H₂ clearance. The flow of compressed air through the head chamber was then stopped, the exhaust ports of the latter closed, and H₂ and O₂ metered into the head chamber was then stopped, the exhaust ports of the latter closed, and H₂ and O₂ metered into the chambers in such a manner that the PO₂ of the atmosphere breathed by the animal remained normal. This was determined by continuously monitoring the PO₂ of the head chamber.

As the recorder pen reached full scale in response to the increasing H₂ concentration at the tip of the electrode, the H₂ and O₂ cylinder valves were closed, the exhaust ports of the head chamber opened, and compressed air turned on with sufficient velocity to quickly remove all H₂ from the chamber. When the recorder pen came back on scale as a result of the now decreasing H₂ concentration in the brain, the flow of air was reduced to a velocity sufficient to sweep exhausted H₂ out of the head chamber in order to prevent its being rebreathed. Simultaneous measurements at the 6 sites were obtained by adjusting the sensitivities of the electrodes using the 10 Kohm potentiometer.

Each clearance curve was replotted on semilogarithmic paper after subtracting the baseline. The half-time for H₂ clearance, in seconds, is easily determined from the semi-log plot and divided into the factor 4158 to yield blood flow in terms of ml/min/100 gm tissue.* No less than 5 clearance curves were obtained from each site at each "sitting," the means of which constituted the values for LCBF for that date.

Vascular Compliance Studies

After the animals were sacrificed, their brains were perfused with iced saline and quickly removed from the cranial cavity. A 10–12 mm segment of the middle cerebral arteries, from the hemorrhaged and un-hemorrhaged sides, were dissected from their vascular beds. Their distal end, along with other secondary branches, were ligated, and a small polyethylene catheter was inserted into their proximal end. The vessels were then simultaneously perfused with iced saline at 100 mm Hg pressure. Differences in elasticity of the 2 vessels were subjectively evaluated by using the operating microscope. Following vascular compliance studies, vessels were prepared for histological evaluation.

Histological Methods

For light microscopic evaluations, vessels were fixed in 10% aqueous formaldehyde and embedded in paraffin so that complete cross sections (6 microns) could be made. Vessels were stained by the following methods: hematoxylin and eosin (H & E) and a silver stain for reticulum, counter stained with periodic acid–Schiff’s reaction. The following procedure was used: 2 vessels were subjectively evaluated by using the operating microscope. Following vascular compliance studies, vessels were prepared for histological evaluation.

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1. The initial site of rupture. Starting at the site of rupture, the vessel was sectioned (6 μ), sequentially with the first and every twentieth section thereafter was stained. This scheme produces a 126 μ distance between the sections that were stained and studied, 1 stained (2–20 kept), 21 stained (22–40 kept), etc. This sequence of staining was occasionally altered, depending upon the presence or absence of damage. The middle cerebral vessels from the unhemorrhaged side (control vessel) was treated the same.

2. For electronmicroscopic evaluation, vessels were

<table>
<thead>
<tr>
<th>Electrode Site</th>
<th>Animal No.</th>
<th>Control</th>
<th>1-6</th>
<th>24</th>
<th>48</th>
<th>240</th>
<th>264</th>
<th>336</th>
<th>744</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus ventralis</td>
<td>437</td>
<td>X</td>
<td>21.4</td>
<td>*13.2</td>
<td>*14.5</td>
<td>*16.3</td>
<td>K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>posterior inferior of thalamus</td>
<td></td>
<td>S.D. 0.8</td>
<td>1.0</td>
<td>1.3</td>
<td>1.6</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
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</tr>
<tr>
<td>Superiolarteral aspect of the geniculate nucleus</td>
<td>443</td>
<td>51.0</td>
<td>2.4</td>
<td>3.3</td>
<td>4.1</td>
<td>6.5</td>
<td>2.7</td>
<td>K</td>
<td></td>
</tr>
<tr>
<td>Nucleus ventralis</td>
<td>443</td>
<td>60.1</td>
<td>*47.6</td>
<td>*58.5</td>
<td>*60.4</td>
<td>*58.7</td>
<td>K</td>
<td></td>
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<tr>
<td>posterior inferior of thalamus</td>
<td></td>
<td>2.0</td>
<td>1.8</td>
<td>4.6</td>
<td>3.5</td>
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<td>Superior frontal gyrus</td>
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<td>62.1</td>
<td>68.5</td>
<td>70.6</td>
<td>No</td>
<td>*76.3</td>
<td>*76.4</td>
<td>*86.4</td>
</tr>
</tbody>
</table>

*Significant change: X 12.8V (ml/min/100 gm tissue) based upon 5 or more sequential measurements.

All data given on sites in animals showing non-significant changes.

A Killed after previously listed measurement.

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perfused with glutaraldehyde and cut into cross-sections at approximately 1 mm thickness. Th pieces were placed in chilled phosphate buffered 4% glutaraldehyde (pH 7.2) for 6 hours, followed by a 12 hour wash in 0.2 M-cacodylate buffered 1% osmium tetroxide, dehydrated in a graded concentration of ethanol, and embedded in Epon 812. Thick sections of tissue blocks were cut subsequently, stained with uranyl acetate and lead citrate and examined in a Phillips EM-300 electron microscope.

To evaluate sites of cerebral electrode placement, brains were fixed in formalin and frozen sections (75 microns) were stained with thionine.

Results

Morphological Changes

Gross Findings

The brains of all animals subjected to subarachnoid hemorrhage within 6 days of sacrifice showed elements of SAH. Basal vessels from Group I animals were slightly less xanthochromic than those of Group II. No thrombosis was evident and there was no brain softening suggestive of infarction in any of the animals.

Microscopic Findings

No mural vascular changes were observed in any of the control vessels studied (vessels in Groups I and II not exposed to rupture or blood injection). Similarly, in vessels exposed to subarachnoid blood injections (Group I), there was no evidence of intimal proliferation or other mural vascular change. However, a considerable number of leucocytic type cells appeared to be adherent to the endothelial lining of some vessels at 16 to 30 days, although this could represent fixation artifact (fig. 1).

In animals hemorrhaged via arterial rupture (Group II), there were various types and amount of mural (subintimal and subendothelial) vascular damage. As expected, all vessels in this group showed more extensive changes at the initial site of rupture. However, the

![Figure 1. Middle cerebral artery from injected-hemorrhaged animal 30 days post-hemorrhage. The medial layer (M) and internal elastic membrane (IEM) is intact, with no evidences of mural vessel damage. Numerous leucocytic type cells (L) appear to be adherent to vessel wall. (H & E) x 430.](http://stroke.ahajournals.org/)

- Figure 1. Middle cerebral artery from injected-hemorrhaged animal 30 days post-hemorrhage. The medial layer (M) and internal elastic membrane (IEM) is intact, with no evidences of mural vessel damage. Numerous leucocytic type cells (L) appear to be adherent to vessel wall. (H & E) x 430.
alterations also occurred both proximal and distal to the point of rupture and appeared to be time dependent. By 3 days following rupture, subendothelial swelling and thickening were present but minimal (fig. 2). These changes were basically limited to the areas immediately proximal and distal to the ruptured site. There appeared to be no cellular infiltration or proliferation in the thickened subendothelial zones (fig. 2). By 6, 10 and 16 days, subendothelial proliferation was much more pronounced and extended further, both proximal and distal, from the point of rupture. (figs. 3, 4 and 5). At 30 days following hemorrhage, subendothelial proliferation, along with other mural damage, became progressively more severe extending, in addition, further from the ruptured site (figs. 6 and 7). The subendothelial proliferation consisted mainly of fibroblasts and smooth muscle cells, embedded in a fine network of reticular and collagenous fibers. Smooth muscle cells were the more predominant element that had increased. By 10 and 16 days following rupture, such endothelial proliferation occupied the entire circumference of the intimal surface and had occluded as much as one-fourth of the vessel's lumen (figs. 4 and 5). The internal elastic membrane, both proximal and distal, showed several points of interruption and evidences of splitting (fig. 4). The medial smooth muscle appeared intact, but showed hemosiderin infiltration, which was also present in the subendothelial proliferative areas. By one month, in addition to the above changes, the medial smooth muscle was shrunken and fibrosed (fig. 6). Hemosiderin pigment was located in both medial and intimal layers of the vessel. With the progressive proliferative activity occurring in the intima, approximately one-half or more of the vessel's lumen was occluded (fig. 6). Regardless of the degree of intimal proliferation, all vessels were found to be patent. There was no evidence of fat or calcium deposits within the intimal proliferation. By 30 days, varying degrees of the above pathological alterations were observed 6–7 mm distal and 2–3 mm proximal from the initial site of vascular rupture.
FIGURE 4. Middle cerebral artery of ruptured-hemorrhaged animal 10 days post-hemorrhage. The internal elastic membrane (IEM) shows points of interruption and splitting (unmarked arrows). Note increase in intimal proliferation (IP). (H & E) X430.

Electronmicroscopy

There was no evidence of morphological alterations in the vessels exposed to subarachnoid blood injection (Group I) or in any control vessels of both groups.

In those vessels hemorrhaged via rupture (Group II), the majority of cells in the subendothelial proliferative areas examined were seen to have ultrastructural characteristics of smooth muscle (fig. 8). Cells were spindle shaped with elongated nuclei. Their cytoplasm contained scattered droplets and variable numbers of thin filaments arranged parallel to the long axis of the cells. Electron-dense areas were seen along these filaments characteristic of attachment points in smooth muscle cells (fig. 8). Pinocytotic vesicles were also observed along the plasma membrane.

Alterations in Vessel Elasticity

In the 2 hemorrhaged groups, there were striking differences in compliance when vessels were subjected to standard intraluminal pressures. In the injected-hemorrhaged group (Group I), no obvious changes in vessel elasticity could be demonstrated when compared to the control of the opposite hemisphere. In the ruptured-hemorrhaged vessels (Group II), there were substantial losses of compliance when compared to control vessels of the opposite side (fig. 9). At the 3 day interval, the decrease in vessel elasticity was of questionable significance, but such changes could be clearly demonstrated by 6, 10 and 16 days, becoming most marked one month following hemorrhage (fig. 9).

Local Cerebral Blood Flow Changes

Sites and values of local cerebral blood flow (LCBF) are shown in the table. Because of the large number of measurements obtained from each site in each animal, no non-significant data are given. We considered changes "significant" only when post-hemorrhage mean blood flow was statistically different from the means of all pre-ruptured control values. Using these criteria no changes in LCBF were observed in the subarachnoid injected-hemorrhaged group (Group I) and flow was altered at only 4 sites in the ruptured-hemorrhaged group (Group II). In the 3 day post-hemorrhaged animal, there was significant
Middle cerebral artery of ruptured-hemorrhaged animal 30 days posthemorrhage. The medial layer (M) is shrunken and the intimal proliferation (IP) of smooth muscle cells have occluded most of the vessel's lumen. (L). (H & E) × 200.

decrease in LCBF in the nucleus ventralis posterior of thalamus for the entire period (table). In the animals followed 10 days post-hemorrhage, there were significant decreases in LCBF in the nucleus ventralis posterior of the thalamus and geniculate nucleus at 1–5 hours, but such changes were only transient, becoming non-significant beyond this time period (table). In the one month post-hemorrhaged animal, the superior frontal gyrus showed significant increases in LCBF occurring late in the time period (264–744 hours; see table).

Discussion

In describing acute vessel constriction seen in the laboratory setting, during operative procedures or even angiographically within hours of subarachnoid hemorrhage, the term vasospasm may be appropriately applied. It must be questioned, however, when used to describe all aspects of the narrowing process, particularly that seen late after SAH. In the present study, SAH was caused by vessel rupture and

Middle cerebral artery of ruptured-hemorrhaged animal 30 days posthemorrhage. Note severe shrinkage and fibrosis of the medial smooth muscle layer of vessel (arrows). Luminal surface of vessel indicated by (L). (H & E) × 430.
was accompanied by morphological alterations similar to those observed in man following SAH. Although angiography was not employed, the mechanisms used in this study to evoke subarachnoid bleeding have been shown also to produce radiographic constriction, the appearance and distribution of which is similar to that in humans associated with aneurysmal rupture. The compliance studies carried out on the hemorrhaged vessels was documentary of the constrictive process.

Subendothelial proliferation, disruption of the internal elastic membrane, and necrosis of the muscular layers of both arteries and veins have all been observed in patients with cerebral infarction secondary to SAH. Similar, though perhaps not identical, changes have been produced by a number of procedures which traumatize the vessel without causing hemorrhage. Such alterations, however, are usually restricted to the site of injury. The principal histological differences described after direct vessel trauma and those that were observed in this study, as well as those from clinical cases, is a quantitative one in that the pattern of alterations is uninterrupted, longitudinal, and found both proximal and distal from the initial site of rupture. In the directly traumatized vessel, the morphological changes involve principally the intima, spare the medial layer, and are localized primarily to the injured site.
The reasons for the diffuse involvement or propagated mural injury that occurs in cerebral arteries following SAH remain unclear. While several hypotheses have been proposed, some of which are strengthened by clinical evidence, none totally explains the morphological alterations seen. Observations of marked morphological changes in arteries found to be constricted on premortem angiograms led Hughes and coworkers to conclude that the acute and severe vasospasm occurring initially following SAH damaged the muscular layer. Alksne described electron microscopic changes in a cerebral artery after the injection of norepinephrine into the subarachnoid space. The acute phase of forceful constriction was concluded to be destructive of muscle cells which was self-propagating and the effect persisted for weeks or longer. While both manipulation of cerebral vessels and the application of blood to their surface is known to provoke an early vasoconstrictory response, morphological changes apparently do not follow these stimuli.

Likewise, distal cerebral infarction, as proposed by Crompton, and stasis of flow would seem unlikely causes of the structural alterations since they have been observed in vessels in which infarction was not a feature. Our initial experiments in primates have discounted blood flow alterations as playing any major role in the production in the pathological alterations of cerebral vessels. The hypothesis that overactivity of the sympathetic-catecholamine system following SAH leads to vascular damage may have merit. In mice, both cardiac necrosis and intramyocardial hemorrhage have been observed after experimental SAH. The heart lesions in these animals were focal and were attributed to the release of catecholamine at nerve endings since reserpine almost totally prevented these changes.

These observations are difficult to interpret, however, since the parasympathetic blocker, atropine, also inhibits heart muscle damage. The consistent findings of brown pigment in the vessel wall of those showing structural changes suggest that it is the dissection of blood into the wall of the artery that is the common factor in the production of the arteriopathy. This hypothesis is strengthened by the clinical observation that symptomatic and angiographic vasospasm is observed principally after vessel rupture in a closed compartment. The mere application of blood or the injection of blood onto the surface of the cerebral vessel does not provoke morphological changes. How the dissection of the blood becomes so widespread and how it produces such diffuse damage is not clear. Neither is it apparent how the abnormal morphology leads to the physiological constriction observed clinically. Perhaps, as shown in this experiment, the morphological alterations cause loss of elasticity at normal intraluminal pressures long before such changes become apparent to light microscopy. The question as to whether the proliferated smooth muscle cells could function normally is also relevant to the concept of vasospasm. This condition could render the vessel hypersensitive to normal physiological responses, resulting in further narrowing of the lumen. Whatever the mechanism, the proliferative changes occurring in vessels after SAH could produce functional and even angiographic correlates, particularly when observed late in the course following SAH.

Acknowledgment

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References

10. Dudley SP, Bevon KJ, Bevon JA: Alteration of inervation and reactivity of monkey cerebral arteries of a subarachnoid hemorrhage. Stroke 8: 10 (abst), 1977
Constrictive endarteropathy following experimental subarachnoid hemorrhage.
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