Morphometry of Brain Parenchymal Vessels Following Subarachnoid Hemorrhage

MICHAEL NOEL HART, M.D.

SUMMARY This study was performed to test the hypothesis that vascular spasm can occur in brain parenchymal vessels following subarachnoid hemorrhage (SAH). Five cc of autologous blood was injected into the cisterna magna of test cats and 5 cc of saline into control cats. After 2 hours, a cranial window was created and cortical tissue frozen in situ with liquid nitrogen. Statistical analysis of 124 test and 93 control arteries and arterioles showed a significantly greater mean wall to lumen ratio in the test vessels but with almost identical mean cross sectional areas for both groups of vessels. This finding provides evidence for at least vasoconstriction, if not frank spasm, in parenchymal vessels in conjunction with SAH.

Methods

Eighteen cats were initially utilized in the experiment. From these, 4 female cats (2 experimental, 2 control, weighing between 2.6 and 2.8 kg each) were selected for morphometric parenchymal vessel analysis because the physiologic parameters of blood pressure, temperature, heart rate, and intracranial pressure (ICP) were continuously monitored and regulated within a narrow range. pH, Pco₂ and Po₂, were determined 3-4 times during each experiment and also were closely controlled (table 1). Each animal was anesthetized with intraperitoneal pentobarbital (30 mgm/kg), intubated and respired with room air. The experimental animals were given 1 cc of 4-6 day-old autologous blood every 30 min for 2 h (5cc) via the cisterna magna by slow injection in order to prevent rapid rises in ICP. Control animals were given artificial CSF by the same dose and route. Fifteen min after the last injection of blood or artificial CSF, ½ inch diameter cranial windows were created bilaterally with a trephine which exposed, but did not violate, the dura. A plastic funnel was placed over each window, and liquid nitrogen was poured into the funnel and left in contact with the dura for 20 sec. At the end of this time the funnel was removed. The frozen brain was removed with the liquid nitrogen-cooled trephine and the removed tissue was placed in liquid nitrogen. Within 1-2 h the frozen biopsies were cryostat sectioned at 6μ, parallel to the cortical surface in order to maximize the number of cross sections of penetrating arteries and arterioles. Sections were stained with ATPase in order to outline the vessels. The stained slides were projected on a screen at a magnification of 500X. All arteries and arterioles found in cross-section were outlined and external diameters and wall thickness (WT) X 2 were measured with a micrometer. Ninety-three control vessels and 124 experimental vessels were measured. From the measurement data, wall to lumen (W/L) ratios and cross-sectional area of walls for each vessel were calculated.

Results

Results are summarized in table 2. The mean external diameter of the arteries from the experimental group is smaller than the control group while the mean cross-sectional areas of experimental and control groups are approximately the same. The mean WT and W/L are significantly greater in the experimental group when compared to the control group by the t-test. When each group is sub-categorized into 2 groups: those vessels greater in diameter than the mean, and those vessels lesser than the mean, the same parameters (WT and W/L) are also significantly different between experimental and control groups with the single exception of WT in vessels less than the mean diameter (table 3). Even distribution of injected blood over the hemispheres was noted in 6 experimental cats terminated early. This was seen as early as 10 min, following a single injection. Conventional aldehyde fixed, hematoxylin and eosin stained sections of the brains showed a significant acute inflammatory infiltrate of the arachnoid space with neutrophils accompanying some of the penetrating cortical vessels into the Virchow-Robin space.
TABLE 1. Physiologic Parameters of Test and Control Animals at Beginning (base) and Termination (end) of Experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>ICP</th>
<th>BP mm Hg</th>
<th>Temp</th>
<th>HR</th>
<th>pH</th>
<th>pCO₂</th>
<th>pO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>base</td>
<td>end</td>
<td>base</td>
<td>end</td>
<td>base</td>
<td>base</td>
<td>base</td>
</tr>
<tr>
<td>Exp.</td>
<td>10</td>
<td>13.5</td>
<td>186</td>
<td>186</td>
<td>35°C</td>
<td>180</td>
<td>185</td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>8.0</td>
<td>200</td>
<td>125</td>
<td>35°C</td>
<td>195</td>
<td>190</td>
</tr>
</tbody>
</table>

TABLE 2. Mean and Standard Deviation for External Diameter, Wall Thickness, Wall to Lumen Ratio and Cross-Sectional Area for Experimental and Control Animals

<table>
<thead>
<tr>
<th></th>
<th>ED</th>
<th>SD</th>
<th>WT</th>
<th>SD</th>
<th>W/L</th>
<th>SD</th>
<th>A</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Expd.</td>
<td>19.85</td>
<td>4.89</td>
<td>5.66</td>
<td>1.31</td>
<td>0.85*</td>
<td>0.63</td>
<td>257.91</td>
<td>107.35</td>
</tr>
<tr>
<td>Total Control</td>
<td>20.40</td>
<td>4.78</td>
<td>5.30</td>
<td>0.86</td>
<td>0.63</td>
<td>0.22</td>
<td>253.67</td>
<td>96.27</td>
</tr>
</tbody>
</table>

ED = external diameter (μm), WT = wall thickness (μm), W/L = wall to lumen ratio, A = cross-sectional area.
*Statistically different from that of controls (p < 0.06).

TABLE 3. Mean and Standard Deviation for External Diameter, Wall Thickness, Wall to Lumen Ratio and Cross-Sectional Area for Experimental and Control Animals Greater than (> ) and Less Than (<) the Mean Diameter for Each group

<table>
<thead>
<tr>
<th></th>
<th>ED</th>
<th>SD</th>
<th>WT</th>
<th>SD</th>
<th>W/L</th>
<th>SD</th>
<th>A</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exptl. &lt; median</td>
<td>16.09</td>
<td>4.81</td>
<td>5.02</td>
<td>1.06</td>
<td>0.98*</td>
<td>0.53</td>
<td>175.70</td>
<td>50.31</td>
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<tr>
<td>Control &lt; median</td>
<td>16.65</td>
<td>1.97</td>
<td>4.85</td>
<td>0.59</td>
<td>0.72</td>
<td>0.18</td>
<td>181.24</td>
<td>38.97</td>
</tr>
<tr>
<td>Exptl. &gt; median</td>
<td>23.60</td>
<td>3.74</td>
<td>6.31*</td>
<td>1.24</td>
<td>0.68*</td>
<td>0.39</td>
<td>337.83</td>
<td>85.42</td>
</tr>
<tr>
<td>Control &gt; median</td>
<td>23.86</td>
<td>3.34</td>
<td>5.82</td>
<td>0.82</td>
<td>0.53</td>
<td>0.21</td>
<td>331.42</td>
<td>86.65</td>
</tr>
</tbody>
</table>

ED = external diameter (μm), WT = wall thickness (μm), W/L = wall to lumen ratio, A = cross-sectional area.
*Statistically different from that of controls (p < 0.06).

Discussion

The results of this experiment show that the brain parenchymal arteries and arterioles of test cats with subarachnoid hemorrhage have smaller external diameters and thicker walls when compared with control animals. The resultant greater W/L ratios in the experimental animals compared with the controls in conjunction with similar mean cross-sectional areas for both groups indicates that the experimental vessels are in a state of constriction compared to the controls. If the experimental vessels were larger (thicker media) in comparison to the control vessels, then they would display greater mean cross-sectional areas in addition to having thicker walls and greater W/L ratios.

The control W/L in the present study is consistent with previously established W/L in the brains of normal cats (0.51) and in ischemic cats exclusive of non-reflow areas (0.58) although vessels in that study were not subcategorized as to size. All physiologic parameters of both test and control animals were similar with the exception of slightly higher ICP in the test animals. However, the highest post-injection ICP recorded was 16.4 mm Hg, a value not considered pathologic.

Autologous 5 day old blood has been previously shown by Watts to cause spasm in meningeal arteries. It is therefore not surprising that penetrating arteries and arterioles of the cerebral cortex should constrict when exposed to necrotic blood for 2 reasons: cortical vessels have access to the arachnoid space because the pial (inner arachnoid) layer accompanies these vessels into the brain creating the Virchow-Robin space. In addition, cortical parenchymal vessels have abundant smooth muscle and adrenergic nerve endings as do meningeal vessels.

The pathogenesis of the vasoconstriction observed in this study is not known but could be due to the same chemically mediated mechanisms which apparently cause spasm of larger cerebral vessels. The observed inflammation in these animals also might be contributory.

In a previous publication it was shown that cerebral vessels can be retained in a physiologic state of contraction by rapid freezing. The present technique represents an improvement over the previous effort as the brain with vessels was frozen in situ in this experiment and in the previous work a brain biopsy was removed before freezing. It is estimated that all of the penetrating vessels sampled in this experiment were frozen within 5 sec of exposure to liquid nitrogen since sections were all taken close to the cortical surface. Other studies which have utilized rapid freezing of brain with a cryoprobe followed by freeze-substitution have in the past established the efficacy of this procedure.
ent experiment allows for the sampling of a much greater number of vessels than is possible with a cryoprobe if samples are taken from near the cortical surface where the tissue freezes most rapidly.

All vessels measured in this study were arranged in cross-section or very nearly in cross-section. In order to preclude a bias which might be introduced into the sample by sectioning vessels at various angles, all external diameters were measured from the widest part of the shortest diameter and the two wall measurements were also taken in this axis (fig.). Thus, all vessels were analyzed as if they were perfect circles. If vessels were not analyzed in this fashion it could be reasoned that one group or the other contained more vessels cut at angles that varied from cross-section and in turn would introduce error in the values obtained for cross-sectional areas.

Others have reasoned that spasm might occur in cerebral parenchymal vessels following subarachnoid hemorrhage. Millikan's\textsuperscript{4} observation that patients with ruptured cerebral aneurysms display neurologic symptoms in the absence of demonstrable spasm of large vessels suggests the possibility of vascular spasm in parenchymal vessels.

In the study of 44 patients with subarachnoid hemorrhage by Kelly et al.\textsuperscript{14} there was no correlation between the presence of vascular spasm and brain perfusion. These results could also be explained by spasm in radiographically undetectable vessels although other explanations also cannot be ruled out.

Grubb et al.\textsuperscript{5} found significant decreases in cerebral blood flow and oxygen utilization in 30 patients with subarachnoid hemorrhage irrespective of whether vasospasm was present or not. In that study there was also a marked increase in cerebral blood volume in those patients with vasospasm. The authors concluded that vasospasm of large vessels might be accompanied by dilatation of parenchymal vessels. It would seem just as reasonable, however, to hypothesize a pooling of cerebral intravenous blood to account for the increased blood volume.

Experimental evidence for intraparenchymal vasoconstriction or spasm is meager because of the technical difficulties in observing these vessels. Most of them are too small to be radiographically evaluated and conventional fixatives are not to be trusted in terms of fixing a vessel at its physiological caliber. In one study by Arano and Sano\textsuperscript{11} subarachnoid hemorrhage was induced in dogs for 3 h followed by perfusion of the brain with carbon black. Gross coronal and microscopic sections of the perfused brains showed good perfusion of the meningeal vessels but very poor perfusion of parenchymal vessels. The authors attributed their results to the "no reflow phenomenon" but did not mention the possibility of parenchymal vessel spasm causing no-reflow. One criticism of that study is the method of perfusing the brain briefly with formaldehyde solution before introducing the carbon black. Since it is known that formaldehyde can cause immediate vasoconstriction,\textsuperscript{12} it is quite possible that failure of the carbon black to perfuse the cortex was an artifact.

The present study does not prove that vasospasm occurs in parenchymal vessels following SAH. It does show, however, that there is a significant constriction of cat brain parenchymal arteries and arterioles associated with the presence of old blood in the subarachnoid space. These findings need to be confirmed by other methods of investigation.

\section*{Acknowledgment}

Susan O'Donnell and Kathryn Sadewasser assisted in the technical aspects of this study.

\section*{References}


\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{vessel.png}
\caption{A vessel sectioned at an angle (A) will appear oval (B) in cross sectional view. Thus the perpendicular line in B represents the true diameter and true wall thickness.}
\end{figure}
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