Reassessment of Cerebral Capillary Changes in Acute Global Ischemia and Their Relationship to the "No-Reflow Phenomenon"

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SUMMARY

Electron and light microscopic studies were performed on rabbit brain to re-examine the structural changes of endothelial cells and perivascular glia following ischemia. Although swelling of perivascular glia occurred, earlier findings of extreme perivascular glial swelling and bleb formation leading to luminal col-

lapse and plugging could not be confirmed. Ischemic brains, however, had a higher proportion of small-diameter capillaries than controls. It is felt that structural changes in ischemic capillary walls in themselves are not sufficient to explain failed cerebral reperfusion, or the no-reflow phenomenon.

Introduction

WHEN BLOOD FLOW to the brain is interrupted for more than a few minutes, vascular changes occur which interfere with the re-establishment of normal flow and these have been referred to as the "no-reflow phenomenon." This phenomenon can be regularly demonstrated in the rabbit after seven minutes of stasis, and becomes progressively worse over the next 30 minutes of stasis. Two quite different mechanisms have been proposed to explain the phenomenon, with evidence presented for each: one is increased viscosity of the stationary blood due to red cell aggregation; the other is narrowing and occlusion of vascular lumina due to ischemia-induced swelling of perivascular glia and, to a lesser extent, endothelial cell swelling and bleb formation.

Prior hemodilution seems to have a pronounced beneficial effect on the reinfusion of a carbon suspension into rabbit brain made globally ischemic for 15 minutes, the time frame within which the "no-reflow phenomenon" is well established. Because this does not seem entirely consistent with an ischemic vascular pathology of occluded capillary lumina, we felt it necessary to re-examine the pathological response of cerebral capillaries to global ischemia. An ischemic time period of 30 minutes was chosen for study to allow for full development of all early pathological changes.

In the earlier histological studies red cell aggregation may have interfered with tissue perfusion fixation. For this reason, we chose to restudy the pathological changes of the ischemic cerebral capillary vasculature in the absence of red cells. At the initiation of total circulatory arrest in rabbits, all vascular changes occurred which interfere with the re-establishment of normal flow and these have been referred to as the "no-reflow phenomenon." This phenomenon can be regularly demonstrated in the rabbit after seven minutes of stasis, and becomes progressively worse over the next 30 minutes of stasis. Two quite different mechanisms have been proposed to explain the phenomenon, with evidence presented for each: one is increased viscosity of the stationary blood due to red cell aggregation; the other is narrowing and occlusion of vascular lumina due to ischemia-induced swelling of perivascular glia and, to a lesser extent, endothelial cell swelling and bleb formation.

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brain, the aorta was cross-clamped and the jugular veins opened. Serum was allowed to flow into the carotid arteries for three minutes. This system achieved almost complete washout of the blood as determined by benzidine staining of coronal brain sections in preliminary animals.

The actual intracarotid pressure achieved in each infusion was calculated using an in vitro model (fig. 1) in which the pressure drop across the entire system (tubing and carotid catheters) was determined for different flow rates. The intracarotid pressure therefore equaled the height of the reservoir (120 cm) minus the pressure drop for the flow rate of the infusion.

Fixation of the brain was then accomplished after 3.5 minutes from the time of aortic cross-clamping in controls (three animals) and after 30 minutes from the time of aortic cross-clamping in the experimental group (three animals). A two-stage infusion fixation was employed, by exchanging the serum reservoir for one containing fixative. One hundred milliliters of a 1% paraformaldehyde, 1.25% glutaraldehyde solution in sodium cacodylate buffer (pH 7.2 to 7.4) were followed by 100 ml of a 2% paraformaldehyde, 2.5% glutaraldehyde solution (pH 7.2 to 7.4), the rate of infusion being recorded so that the intracarotid pressure could be calculated.

After removing the brain, 0.5 to 1.0 mm³ tissue blocks were cut from frontal cortex, inferior hippocampus, anterior basal ganglia and thalamus, and were fixed an additional hour. The blocks were washed in Sabatini's washing solution, post-fixed in Dalton's chrome osmium and 1% uranyl acetate and dehydrated in increasing concentrations of acetone. The tissues were infiltrated in propylene oxide and embedded in an epon araldite mixture. Thin sections of a single block from each area were examined with a Philips EM 300 electron microscope. From the same tissue block, 1-μ thick sections stained with toluidine blue were examined with a light microscope.

Each entire 1-μ thick section was evaluated in the following manner. Every third microscopic field in a lattice pattern was photographed at 200X magnification. For each section a 0.01-mm calibration slide was similarly photographed. The negatives were projected at 12.5X magnification using a photographic enlarger. The diameter of each vessel in every photograph was measured and the frequency distribution of capillaries was calculated. In addition, the percentage of vessels showing pericapillary glial swelling was calculated. The frequency distributions of capillary sizes between the two groups of animals were compared using the Kolmogorov-Smirnov test for significance of greatest difference between two cumulative proportions.

Results

In brains from control animals, fixed immediately after serum infusion and within 3.5 minutes of aortic cross-clamping, most of the vessels were normal (fig. 2). Less than 8% of the capillaries had swollen perivascular glia and the neuropil appeared entirely normal. However, in animals subjected to 30 minutes of ischemia 54% of the vessels showed extensive swelling of perivascular glia. A representative example is shown in figure 3. The frequency of perivascular glial swelling did not vary in the different areas of brain examined. The lumina of vessels retained the normal round configuration seen in intravascular perfused brain even when surrounded by swollen glia and there was no evidence of luminal collapse. Astrocytic swelling was not limited to perivascular glia, but was also seen in areas which appeared to be distant from blood vessel walls. There was no evidence of endothelial cell swelling or bleb formation. Endothelial tight junctions remained intact (fig. 3) and there was no evidence of interstitial edema.
Figure 3. Electron micrograph of capillary in frontal cortex after 30 minutes' ischemia. Gliarial end feet (G) and astroglial process (A) are swollen. The intracellular organelles are dispersed and the intervening cytoplasm is electron lucent. The interstitial space is unchanged from 3.5 minutes' ischemia. The insert is an enlargement of an intact tight junction between two endothelial cells. Markers are 1 μ.

Figure 4 shows the frequency distribution of capillary diameters for both the control animals and those subjected to 30 minutes of arrest when all four areas of the brain were considered together. Neither group had capillaries smaller than 3 μ in diameter. The shift in the combined frequency distribution of ischemic brains toward capillaries of smaller size is significant (p < 0.001) for each area of brain (frontal cortex, hippocampus, basal ganglia, thalamus) when evaluated separately.

The range of infusion rates was between 10 and 27 ml per carotid per minute. The perfusion pressures ranged between 65 and 110 cm H₂O. The mean infusion rates and pressures did not differ with statistical significance between the control and ischemic groups.

Discussion

In the present studies a moderate degree of perivascular gliarial swelling was found after 30 minutes of global ischemia in rabbits. Contrary to the studies of Chiang et al., which were used to explain failed cerebral reperfusion or the "no-reflow phenomenon" following 15 minutes of ischemia, we did not find severe gliarial swelling with profound capillary collapse, endothelial cell swelling or bleb formation. Narrowing of capillary lumina occurred in the current experiments as measured in perfusion fixed specimens, the distribution of capillaries being more heavily weighted toward the small capillary sizes in ischemic as compared to non-ischemic rabbits. Only a small number of vessels in the 3 to 3.9 μ range were present in both ischemic animals and controls, but their presence in both groups would presumably mean that they are large enough to permit passage of normal red cells. In addition, the vascular changes appeared evenly distributed throughout the areas of brain studied and did not show sparing of superficial cortex in relation to basal ganglia and thalamus which characterizes the "no-reflow phenomenon."

The findings that endothelial cells did not swell or form blebs, that tight junctions were preserved and that there was no evidence of interstitial edema imply that the blood-brain barrier, at least to large molecules, was unaffected by the ischemia, as proposed by Olsson et al.

It is reasonable to assume that the observed perivascular gliarial swelling in the ischemic brains resulted in the shift in distribution of capillaries to smaller sizes. Another explanation for this shift, however, is reduced intracapillary pressure due to either shunting or precapillary vessel narrowing. Wade et al. have suggested that cerebrovascular resistance increases during ischemia because of a rise in extracellular potassium concentration which depolarizes smooth muscle cell membrane causing vascular smooth muscle contraction.

The differences between the present experiments and those of Chiang et al. are probably attributable to differences in the flow properties of the perfusing fixative. Although Chiang et al. perfused fixative through the common carotid arteries from a reservoir 160 cm above the animal, they did not mention taking into account the pressure drop across the carotid needles. Since the pressure drop across a fixed resistance is a function of the flow rate and since high flow rates were required in their experiments to perfuse both the internal and external carotid vascular beds, the pressure drop across their carotid needles must have been considerable. In our experiments, we restricted the perfusion to the much smaller internal carotid artery...
Introduction

FISHER1, 2 mentioned his experience with the autopsy findings in 200 cases of cerebrovascular accident clinically suspected of having middle cerebral artery (MCA) thrombosis and the University of Minnesota Department of Radiology, Minneapolis, Minnesota 55417.

SUMMARY Radionuclide cerebral blood flow (CBF) examinations of 48 patients with atherosclerosis, 18 with occlusion and 30 with stenosis of the internal carotid artery (ICA) were correlated with their respective cerebral angiograms.

The following results were obtained. Flow was usually unilaterally diminished in 29 (60%) of 48 patients, including 14 (78%) with occlusion and 15 (50%) with stenosis. Sixty-two percent of the subjects with severe stenoses and 46% of the patients with mild stenoses had a positive flow study. Diminished flow was evident in the neck in 80% of the patients, intracranially in 20%. Positive radionuclide angiograms always pointed to the side with occlusion or the greater degree of stenosis even though bilateral internal carotid disease was frequently found (54%). The data leading to the differentiation between major and minor ICA stenosis are not sufficient to justify any conclusion.

Radionuclide Cerebral Blood Flow and Carotid Angiogram

Correlation in Internal Carotid Artery Disease

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References

Reassessment of cerebral capillary changes in acute global ischemia and their relationship to the "no-reflow phenomenon".
E G Fischer, A Ames, 3rd, E T Hedley-Whyte and S O'Gorman

*Stroke*. 1977;8:36-39
doi: 10.1161/01.STR.8.1.36

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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