Impaired microvascular filling after focal cerebral ischemia in monkeys

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Impairment of microvascular filling was demonstrated in relation to focal cerebral ischemia in the monkey. Temporary or sustained middle cerebral artery (MCA) clipping was achieved with a microsurgical technique. Animals were sacrificed by perfusion with a carbon black suspension. Brains were fixed in formalin, and the extent of microvascular carbon filling was estimated grossly and microscopically. In most animals, MCA occlusion of 2 hours to 7 days produced diminished filling in small vessels in the MCA territory of supply. The impairment of filling was most pronounced in the deep subcortical structures but also affected the cortex in some animals. Temporary and sustained occlusion of equal duration produced roughly equivalent areas of abnormal filling. The impairment of vascular filling tended to be more extensive with increasing duration of occlusion. Hypotension during MCA occlusion caused almost total non-filling of the microvasculature in the entire MCA territory. Impaired filling of vascular channels may play a role in the pathogenesis of some clinical cerebrovascular diseases.

KEY WORDS cerebral microvasculature  focal ischemia  experimental stroke

It is generally believed that the permanent brain injury which may follow transient ischemia is due to the susceptibility of neurons to oxygen deprivation. Recent investigations have suggested that early obstructive changes in the cerebral capillaries may also be a major factor limiting recovery from ischemia. By using a technique of carbon-black perfusion, Ames and co-workers demonstrated the nonpatency of cerebral capillaries immediately after complete arrest of the cerebral circulation of more than 5 min. This “no-reflow” phenomenon implies an inadequate recirculation of blood after transient ischemia.

The obstruction of the microcirculation following global ischemia is probably due to increased blood viscosity and occlusive changes in and around the terminal blood vessels. Chiang, et al., presented electron microscopical data which indicate that swelling of the endothelium and the perivascular glial cells occurring during ischemia may narrow the capillary lumen to a fine slit. Similar ultrastructural changes have been described in cardiac muscle after ischemia. It has also been shown that these vascular changes may be influenced or abolished by appropriate treatment. The survival time of the brain can be considerably prolonged if secondary vascular changes can be reversed.

The properties of the no-reflow phenomenon have until now been studied only in experiments with virtually complete arrest of the cerebral circulation. Impaired vascular
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filling in relation to focal cerebral ischemia, in which leptomeningeal collateral supply plays a protective role, has not yet been demonstrated with the carbon-black perfusion technique. However, it is known that permanent occlusion of the middle cerebral artery causes changes in the pial microcirculation with hemocoagulation, sludging, and eventual failure of the collateral circulation. Improper vascular filling might be expected under such conditions.

The study we are reporting is part of a series of investigations of focal ischemia and was undertaken to elucidate some properties of vascular filling following focal cerebral ischemia. The results have also been correlated with previously reported clinicopathological findings.

Method

Fifty adult monkeys (Macaca mulatta) were used for this study. The animals were anesthetized with Phencyclidine hydrochloride (10 mg/kg intramuscularly) and sodium pentobarbital (up to 30 mg/kg intravenously or intraperitoneally). A retro-orbital microsurgical approach, originally described by Sundt and Waltz, was used to expose the origin of the middle cerebral artery (MCA) in minimally traumatic fashion. The vessel was then permanently or temporarily occluded with a Scoville aneurysm clip. Durations of MCA occlusion are presented in Table 1. Four nonoperated monkeys served as controls. In six monkeys, the effect of simultaneous MCA occlusion and hypotension on microvascular filling was assessed. Aortic blood pressure was monitored with an aortic catheter, a Statham strain gauge, and a Honeywell Visicorder. In these monkeys, removal of 60 to 120 cc of arterial blood lowered the systolic blood pressure to 30 to 40 mm Hg.

At predetermined intervals (1/8, 1/8, 2, 4, and 24 hrs, and 7 days) the animals were reanesthetized, and the jugular veins and descending aorta were exposed. A heparinized catheter with an inner diameter of about 3 mm was threaded into the thoracic aorta through an opening in the abdominal aorta. Animals were then artificially ventilated, the chest was opened, and the aorta was clamped immediately above the heart. Simultaneously the jugular veins were cut, and perfusion was begun. In monkeys sustaining temporary occlusion, the clip was removed from the MCA less than 2 min before perfusion. This brief time interval was achieved by removing the clip after insertion of the aortic catheter but before opening the chest. In animals undergoing permanent MCA occlusion, the clip remained in place during perfusion.

The initial perfusate was a mixture of 10% formalin and colloidal carbon. This carbon suspension is prepared especially for biological use and, unlike India ink, it is free of shellac, which may cause clotting of blood. The carbon suspension is composed of 10% soot, 9.5% gelatin, and 1.3% phenol. Carbon suspension and formalin were placed in separate perfusion bottles about

* Supplied by Pelikan Werke, Guenther Wagner, Hannover, Germany.

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<th>Extent of Impaired Vascular Filling</th>
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<td>Total</td>
<td>3</td>
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* This group is simply a lumping of the "permanent" and "temporary" groups. For example, all animals subjected to 2 hrs of occlusion of a permanent or temporary character are considered together here.

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140 cm above the chest of the animals. These bottles were connected by separate plastic tubing assemblies to a Y-adaptor on the aortic catheter. Perfusion with both solutions was continued for 2 min and was then followed by perfusion with carbon suspension alone for 2 min.

Brains were removed and additionally fixed by immersion in 10% formalin for 2 to 4 days. Consecutive coronal sections 3 to 5 mm thick were cut and inspected microscopically. Tissue blocks were surveyed under a dissection microscope (10 to 40× magnification) for areas of improper vascular filling. Thinner slices were cut with a tissue sectioner (Smith-Farquhar) and cleared in glycerin. These sections were mounted in 50% glycerin in water and used for light microscopic observations. In a few animals, paraffin sections were prepared and stained with the luxol fast blue-PAS technique.

The extent of poor filling was graded according to a 4-point scale (Crowell, et al.):

0 No abnormal vascular filling
1 A few microscopical foci of poor filling not exceeding 3 mm in diameter
2 Medium-sized area of poor filling usually confined to basal ganglions
3 Large cortical and subcortical area of impaired filling in the territory of the MCA.

Results

Distribution of Carbon in Normal Brain Tissue

In control animals, macroscopic inspection revealed even penetration of carbon throughout the brain vasculature (Fig. 1 left). The gray matter was considerably darker than the white matter since the capillary density of the latter is lower (Fig. 1 right). Low-power light microscopical observations showed that practically all vessels, even in their finest ramifications, were well filled with carbon. The control hemisphere of most animals subjected to occlusion of the MCA also showed even and complete filling of vessels. In several of these cases, however, a few scattered areas of cortex were poorly filled. Undetected hypoxia or improper perfusion technique (poor placement of perfusion catheter, incomplete aortic clamping, etc.) may have given rise to the impaired vascular filling seen in a few control hemispheres.

Distribution of Carbon in Brain Tissue after Occlusion of Middle Cerebral Artery

Impaired Vascular Filling in Monkeys with Permanent MCA Occlusion. Macroscopically, impaired vascular filling manifested itself as a patchy, irregular pale gray coloration standing out in sharp contrast to adjacent and contralateral areas of normal blackening (Fig. 2 upper). Under the dissec-
Impaired vascular filling was observed in deep subcortical structures in animals subjected to 2 to 4 hr MCA occlusion; macroscopic evidence of infarction was not yet apparent. In many of these brains, the internal capsule and periventricular caudate nucleus showed little or no evidence of abnormal vascular filling (Figs. 2 lower left and 3 upper). Previous work has demonstrated that these areas frequently escape infarction after temporary MCA clipping for 2 to 4 hrs. Several temporal features of impaired filling during permanent MCA occlusion were suggested (Table 1). All three animals with 2-hr clipping (the shortest clipping interval) showed some evidence of poor filling, and
two of these animals showed Grade 2 to 3 changes. Animals subjected to 4 to 24 hrs of occlusion tended to have more extensive poor filling, but these differences were not statistically significant.

**Impaired Vascular Filling in Monkeys with Temporary MCA Occlusion.** Macroscopic damage and intravascular thrombosis in the proximal MCA were not observed in monkeys subjected to temporary MCA occlusion. Despite the fact that anterograde flow was restored in the MCA, a high proportion of animals clipped for 2 to 24 hrs showed signs of poor vascular filling (Table 1). The macroscopical and microscopical features of these changes were qualitatively identical to abnormal filling observed in animals with permanent occlusion of the MCA (Figs. 3 and 4).

The extent of poorly filled vessels was loosely correlated with duration of occlusion; extent of impaired filling tended to increase with increasing duration of occlusion (Table 1). After 15 min of temporary occlusion, all animals showed little evidence of impaired vascular filling, but most animals with 2 hrs to 7 days of occlusion showed a widespread filling abnormality. Chi-square tests showed that the extent of filling abnormality after $\frac{1}{2}$ hr of temporary occlusion was different from that after 2, 4, and 24 hrs of temporary occlusion ($p \leq .05$).

**Temporary and permanent occlusion of the same duration appeared to produce poor filling in lesions of comparable dimensions (Table 1).** When data for these two groups are considered together, the correlation between increasing extent of poor filling and increasing duration of occlusion becomes somewhat more apparent. Chi-square tests showed that the extent of filling abnormality after $\frac{1}{2}$ hr of occlusion was different from that after 2, 4, and 24 hrs, and 7 days ($p \leq .01$).

**Impaired Vascular Filling in Animals with Temporary Occlusion of the MCA and Simultaneous Hypotension.** Animals in this group manifested extremely severe abnormal filling. Areas of nonfilling were unequivocally white to the naked eye, and microscopic examination revealed that almost all vessels in the affected area were devoid of carbon. The extent of impaired filling was also striking. All six animals had extensive poor filling (three Grade 2, three Grade 3), and two of these animals showed impaired filling in virtually the entire subcortical and cortical territory of the MCA. This magnitude of impaired vascular filling was never observed in nonhypotensive animals.

**Discussion**

The results show that in the monkey transient or sustained focal cerebral ischemia can lead to a focal impairment of filling in small vascular channels. Such improper vascular filling may well play a role in some cerebrovascular disease states in man.

The vascular abnormality described appears to be a counterpart to the "no reflow" phenomenon observed by others in global cerebral ischemia. The term "no reflow" seems inappropriate for our findings, however, since MCA clipping does not in fact totally eliminate distal blood flow, and thus there is no possibility of "reflow" in the strict sense. Furthermore, in cases of permanent MCA occlusion, flow restoration or
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enhancement was not attempted. We shall avoid the term "no reflow" here and refer simply to an impaired filling of small vessels after focal ischemia.

The topographical distribution of diminished vascular filling after focal ischemia suggests the phenomenon may be important in the pathogenesis of cerebral infarction. In every instance, improper filling of vessels was most pronounced in the basal ganglia and less striking in neocortical structures. Cerebral infarction after MCA occlusion in the monkey is also most extensive in subcortical areas, and infarction and poor filling of vessels were coextensive in the present study. These data suggest that the two phenomena are closely related, and it seems likely that the vascular abnormality is a causative factor in the production of infarction.

The precise temporal characteristics of abnormal vascular filling after focal cerebral ischemia cannot be derived from the present data. The variability of the phenomenon would require more animals in each experimental group in order to achieve statistically significant differences throughout; nonetheless, the results reported show that widespread poor vascular filling can occur as soon as 2 hrs after vascular occlusion and that the abnormality tends to become more severe over the interval 2 hrs to 1 week. Other studies have shown that the clinical deficit is minimal after 2 hrs of temporary MCA occlusion, and the infarction is mild to moderate after this duration of temporary clipping. Thus, impaired vascular filling, like failure of cortical collaterals, probably antedates widespread irreversible tissue necrosis and may therefore play a role in causing cerebral infarction.

Failure of adequate collateral circulation and impaired vascular filling appear to be closely associated after focal cerebral ischemia. Abnormal filling is most striking in subcortical areas where collateral is thought to be relatively poor. Infarction, which is held to be a result of failed collateral supply, is likewise most prominent in the basal ganglions after MCA occlusion. Hypotension, which aggravates failure of collateral supply, and causes more extensive infarction after MCA occlusion, produces extremely severe and widespread nonfilling after MCA occlusion.

Data from other workers shed some light upon the intimate mechanisms by which poor vascular filling may be engendered during focal ischemia. Chiang, et al., have provided electron microscopical evidence that endothelial and glial swelling can narrow capillary lumina to slits after global cerebral ischemia. In focal cerebral ischemia, Meyer has provided in vivo observations of sludging, hemococoncentration, and small vessel stasis, together with postmortem histological evidence of endothelial swelling, cerebral edema, and perivascular cellular infiltrates. Thus, it is possible that increased microcirculatory viscosity and swelling of the microvascular endothelium and perivascular elements are responsible for the observed abnormality in the filling of small vessels after focal cerebral ischemia.

Prevention of poor microvascular filling might be useful in the therapy of cerebral ischemia and infarction. Studies on the remediability of improper microvascular filling after focal ischemia are to be presented elsewhere.

Summary

The effect of temporary and sustained occlusion of the middle cerebral artery (MCA) on microvascular filling has been assessed in the monkey by a carbon-black perfusion technique. In many animals, MCA clipping of 2 hrs to 7 days produced diminished filling of small vessels in the MCA territory of supply. Vascular lesions were most pronounced in the deep subcortical gray matter, but the cortex was also affected in some animals. Temporary and sustained occlusion of equal duration produced comparable degrees of abnormal microvascular filling. The abnormality tended to become more extensive with increasing duration of the occlusion. Hypotension during focal ischemia caused almost total nonfilling of the microvasculature in the entire MCA territory. Impaired filling of the microvascular channels may play a role in the pathogenesis of some clinical cerebrovascular disease states.

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