period. It may be that a pointed stump configuration does not persist after several weeks, but the present study did not examine chronic occlusion configuration.

While we have assumed that the artery became occluded simultaneously with the recent stroke, several reports have indicated that symptoms of cerebral ischemia, including stroke, can continue to occur subsequent to occlusion of the artery. Clinicians often find themselves evaluating the angiographic demonstration of an occluded artery in a recently symptomatic patient and having to decide if the occlusion is recent or remote. Our results suggest that the angiographic appearance of the proximal end of the occlusion may not accurately predict the age of the occlusion in the first 6 days from stroke onset, although a pointed appearance was most often seen.

Regardless of the configuration of the proximal carotid occlusion, another important parameter in suggesting a recent occlusion is the occurrence of retrograde flow of contrast material from the ophthalmic artery down to the cervical internal carotid. Selected patients with stroke having this condition have benefited from acute surgical restoration of cerebral blood flow.

It should be emphasized that prolongation of the angiographic arterial phase to at least 4 sec is important to determine if there is delayed antegrade flow through an apparent, rather than a real, complete carotid occlusion.

References

Correlation Between rCBF and Histological Changes Following Temporary Middle Cerebral Artery Occlusion

Akira Tamura, M.D., Takao Asano, M.D., and Keiji Sano, M.D.

SUMMARY Correlations between changes in regional, cortical, cerebral blood flow (rCBF) and histological changes in the corresponding brain regions were examined following middle cerebral arterial occlusion in 24 cats. In all animals, the duration of arterial occlusion was 2 hours followed by 2 hours of recirculation. The animals were divided into 2 groups according to the severity of the observed histological damage. Severe cortical damage was observed in 8 cats (Group A), and, in the remaining 16 cats, little or no cortical damage was seen (Group B). There was a statistically significant difference between these 2 groups in the average rCBF values during ischemia. During recirculation, there was a prompt and uniform recovery of rCBF in animals in group B but a marked diversity of rCBF ranging from hyperemia to oligemia in animals in group A. This diversity of rCBF reflects inhomogeneous blood flow. This study indicates potential hazards for surgical revascularization in the acute stage of stroke when brain damage has progressed beyond a certain level.

Since the development of microsurgical techniques, reperfusion of brain distal to an occluded artery and the possibility of its clinical application following an occlusion of a major cerebral artery have been widely investigated. In most instances techniques such as superficial temporal-middle cerebral artery (STA-MCA) anastomosis have been carried out in the chronic stage of stroke with favorable results reported. However, in acute stroke early surgical revascularization with STA-MCA anastomosis or embolectomy of occluded cerebral arteries has seldom been done for fear of causing either an intracerebral hemorrhage or an aggravation of cerebral edema. Occurrence of intracerebral hemorrhage following revascularization of an occluded proximal artery in man and the detrimental effects of post-ischemic hyperemia in animals after stroke have both been reported.

In the present study the influence of early revascularization was investigated hemodynamically and histologically using an animal with regional cerebral ischemia to simulate the efforts of surgical revascularization following proximal arterial occlusion. The inherent technical difficulties of a hemodynamic study in a stroke model are related to the fact that regional cerebral blood flow (rCBF) is
subject to considerable variation even under normal conditions. With ischemia reduction of rCBF is not homogeneous because of anatomical variations of collateral channels, and inhomogeneous flow in microcirculation occurs, a condition known as patchy non-filling in the ischemic cortex. This inhomogeneity of blood flow in an ischemic cortex would be expected to cause different degrees of cell damage in neighboring cerebral tissues which, on recirculation, might result in an even more enhanced inhomogeneity of cortical blood flow. The microregional analysis of blood flow has not been possible with conventional rCBF studies which use isotope clearance methods. To cope with this problem, we have used hydrogen electrodes to measure regional blood flow in very small areas of cerebral cortex. Regional histological changes around each hydrogen electrode were examined after recirculation for 2 hours, so that the relationship between rCBF and the histological change in the same area of cerebral cortex could be evaluated. The aim of the present study is 2-fold: 1) to investigate the inhomogeneous nature of microregional cerebral blood flow during ischemia and recirculation, and 2) to explore the correlation between the microregional blood flow changes and the histological changes in the corresponding areas of cerebral cortex.

Methods

Twenty-four adult cats of both sexes weighing between 2.9 and 4.0 kg were anesthetized with halothane (1 to 2%) and tracheostomized. Arterial and venous catheters were inserted into the femoral artery and vein. The animals were then paralyzed with gallamine triethiodide (2 mg/kg) and mechanically ventilated with a respirator (Aika R-60). Respiratory conditions were adjusted to maintain the PaCO₂ between 30 and 40 mm Hg and PaO₂ between 90 and 130 mm Hg by intermittent blood gas determinations (ABL-1). Base excess greater than -4 milliequivalents per liter was corrected by intermittent intravenous infusion of 7% sodium bicarbonate. The systemic arterial pressure was continuously monitored. The esophageal temperature was maintained around 37.5°C by the use of a heating blanket and an infrared electric lamp. For the maintenance of anesthesia 1-2% of halothane was continuously given during the experiment.

With the skull fixed in a stereotaxic holder, the horizontal portion of the right middle cerebral artery (MCA) was exposed by the transorbital approach under an operating microscope. The MCA was occluded at the lateral margin of the optic nerve by a clip with a short and straight blade. Special attention was paid to avoid any subarachnoid hemorrhage or surgical trauma to the cerebral surface. The dural defect was packed with gel-foam and cotton plugs to prevent leakage of cerebrospinal fluid. Two hours after application, the clip was removed and the brain was recirculated for 2 hours.

Regional Cerebral Blood Flow

For the measurement of rCBF, 2 or 3 Teflon-coated platinum wires with bared tips of 0.5 mm in length and 0.3 mm in diameter were stereotaxically inserted through small burr holes in the skull into predetermined positions in the middle supra-Sylvian and middle ecto-Sylvian gyri on the right side of the brain, i.e., A 9.0 and A 14.0, according to Snyder’s atlas. A reference electrode of Ag/AgCl was placed subcutaneously. Hydrogen gas was given directly into the endotracheal tube in a concentration of about 10 vol% for 3–5 minutes. The rCBF values were calculated using the 2 minute initial slope index. The measurement of rCBF was repeated every 30 minutes during the 2 hours of MCA occlusion and during the 2 hours of recirculation.

Neuropathology

The brain was perfusion-fixed by infusion of physiological saline followed by Karnovsky’s solution via the thoracic aorta at a pressure of 120 mm Hg. Thirty minutes prior to the perfusion 10 ml of 2% Evans blue (EB) solution was given intravenously. After immersion in 10% formalin for a week, the brain was sectioned in the coronal plane, i.e., A 9.0 ± 0.5 mm, A 14.0 ± 0.5 mm, and A 19.0 ± 0.5 mm, according to Snyder’s atlas, and inspected for leakage of EB and shift of midline structures. Histological studies were carried out using serial sections around electrodes stained with hematoxylin-eosin and Klüver-Barrera (Nissl-myelin) stains.

Results

There was no significant change in the systemic arterial pressure and arterial gas tension before, during the MCA occlusion, or during the recirculation period in the 24 cats (table 1).

Severe damage in the cerebral cortex, subcortical white matter and basal ganglia was observed in 8 of 24 cats. In these 8 cats, the gyri on the ischemic side were broadened and the midline was shifted (fig. 1). The gray matter was swollen and the majority of neurons were found to be shrunken and triangular with dark-staining nuclei (fig. 2). These histological changes were present only in the portions of the brain supplied by the occluded MCA (fig. 3). In 3 of these 8 cats cortical damage was accompanied by hemorrhagic infarction with localized or diffuse microscopic petechial hemorrhages. In 4 of these 8 cats there was a marked leakage of Evans blue (fig. 1).

In the remaining 16 cats little or no cortical damage was observed. The brains were of normal size and the fixation by perfusion caused a uniform hardening. No midline shift was noted. Neither leakage of EB nor hemorrhage was present. The neurons had a normal appearance and no ischemic or dark cell changes were seen in most of the areas inspected. In 5 cats, in small spotty areas not exceeding 3 mm in diameter, there were some neurons which were slightly shrunken with
CORRELATION BETWEEN rCBF AND HISTOLOGICAL CHANGES/Tamura et al. 489

TABLE 1  Systemic Arterial Pressures (SAP) and Blood Gases Before, During MCA Occlusion, and During Recirculation in Animal Groups A and B

<table>
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<tr>
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<th>Before</th>
<th>During MCA occlusion</th>
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<td>SAP  (mm Hg)</td>
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<td>138.7 ± 16.7</td>
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<td>pH 7.394 ± 0.076</td>
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<td>O2 34.0 ± 2.7</td>
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<td>BE -3.9 ± 2.5</td>
<td>-2.6 ± 3.3</td>
<td>-3.7 ± 2.3</td>
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</table>

( mean value ± se)

Table 1 shows the systemic arterial pressures (SAP) and blood gases before, during MCA occlusion, and during recirculation in animal groups A and B. The values were acquired before, during, and after MCA occlusion.

The correlation between rCBF and histological changes was analyzed as follows. The rCBF values from each hydrogen electrode were grouped according to the histological findings in the cerebral cortex surrounding each electrode, i.e., about 1 mm from the tip of each electrode (table 2). Values from electrodes which were inserted into the border of the infarcted areas were discarded. The time courses of the average rCBF values from the 15 hydrogen electrodes in the severely damaged cortex of 8 cats (Group A) and of the 31 electrodes in the normal or slightly damaged cortex in 16 cats (Group B) are shown in fig. 4.

The average rCBF before occlusion was 45.4 ± 2.3 ml/100 gm/min (mean value ± se) in group A and 46.5 ± 1.6 ml/100 gm/min in group B, showing no statistically significant difference. The average rCBF values during the ischemic period were, however, significantly different (p < 0.01). In group A average rCBF values during MCA occlusion were only 6.8 ± 0.9 ml/100 gm/min, and in group B they were 25.3 ± 0.8 ml/100 gm/min. Following release of the clips, there was a prompt and uniform recovery of rCBF in the post-ischemic period in group B (fig. 4), the final average rCBF being 46.8 ± 3.8 ml/100 gm/min. In group A, there was a pronounced diversity in the rCBF values during post-ischemic recirculation (fig. 5). Because of this diversity, 2 subgroups were formed according to the presence or absence of Evans blue leakage (fig. 4). In the subgroup with leakage of EB, the average rCBF value before injection of EB was 40.0 ± 8.2 ml/100 gm/min. In the subgroup with no leakage of EB, the value was only 6.2 ± 3.6 ml/100 gm/min (fig. 4). This difference was statistically significant (p < 0.01).

The average rCBF values at each electrode of group A and B during the MCA occlusion are shown in figure 6. It is apparent that the severity of cortical damage was related to the degree of rCBF reduction during the ischemic period. The critical values of rCBF for irreversible cortical damage in the 2-hour MCA occlusion are considered to lie between the lowest value of group B and the highest value of group A, i.e., around 12-15 ml/100 gm/min.

moderate astrocytic swelling. These changes were more prominent in the floor of sulci and/or basal ganglia (fig. 3).

Histological studies of ischemic cerebral cortex revealed 2 distinct patterns of morphological changes: one essentially normal (Group B) and the other severely damaged (Group A).

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FIGURE 2. Representative histological appearances of the middle supra-sylvian gyrus from a rat in group A animals (A) and another in group B animals (B). The neurons in (A) are shrunken and triangular with dark staining nuclei, and the neuropil is edematous, whereas the neurons and neuropil in (B) are normal. Hematoxylin and eosin, X 520.

Discussion

The reversibility of brain damage following an ischemic insult depends on the duration as well as on the severity of the ischemia. Several reports have dealt with the maximum permissible duration of regional ischemia using MCA occlusion models, indicating that the maximum duration of ischemia compatible with the recovery of brain function is 4 to 6 hours when followed by recirculation. The severity of the CBF reduction during ischemia and subsequent changes of the CBF during recirculation have not been previously studied. The variability of rCBF following permanent MCA occlusion has been demonstrated in experiments reported by Blair et al., Sundt et al., and Heiss et al. These studies point out the importance of measuring rCBF during both ischemia and recirculation. It is speculated that the inhomogeneity of blood flow reduction during ischemia results in an inhomogeneity of cortical damage and thus may affect the course of rCBF during recirculation. Measurement of the variability of rCBF during and after
ischemia using the MCA occlusion model is relevant to an investigation of the reversibility of brain damage following ischemia.

The histological criteria of irreversible cerebral damage during acute ischemia remain controversial. In this study, the histological examination of the cerebral cortex revealed 2 distinct patterns. In one group, there was prominent leakage of EB, hemorrhagic infarction and edema. The microscopic investigation of cortex in this group of animals revealed typical ischemic and dark cell changes with the majority of neurons shrunken and triangular with dark-staining nuclei. These changes were detectable only within the part of the brain supplied by the occluded MCA and most marked at the depths of sulci. Such severe damage to cortical structure suggests irreversible cell damage, i.e., cerebral infarction. In the other group, light microscopic examination revealed no ischemic cell changes except in 5 cats, where there were mild ischemic changes in some neurons distributed in small, spotty areas in the depths of sulci. The reasons for a clear-cut morphological difference between group A and B may be recirculation, which has been reported to exert either a beneficial or detrimental influence on the histological evolution of brain damage from ischemia. The use of halothane as an anesthetic agent, although known to exacerbate brain damage from ischemia, is not likely to have been responsible since Crowell et al., Sundt et al., and Shintani et al., using pentobarbital and/or phencyclidine hydrochloride, have independently reported the occurrence

### Table 2

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(mean value ± SE).

**Figure 4.** The time course of the mean rCBF values in the severely damaged cortex in group A animals (left) and the normal or slightly damaged cortex in group B (right). The average rCBF values in the recirculation period in group A are compiled according to the presence (closed circle) or absence (open circle) of the leakage of Evans blue.
of cerebral infarction in about one-third to one-half of animals with ischemia similar to the present study.\(^\text{12, 14, 30}\) Combining both small spotty (5 cats) and large infarctions (8 cats) there is approximately a 50 percent incidence of infarction, not significantly higher than in those reported experiments. There were correlations between the cortical histological changes, the severity of rCBF reduction during ischemia and the post-ischemic courses of rCBF during recirculation. In group B animals, with only slight or no cortical damage, rCBF showed a uniform recovery pattern, implying that the cerebral cortex in group B animals did not sustain much damage from ischemia and recirculation had a beneficial effect on recovery.

In group A animals there was an extreme diversity in the course of rCBF during the recirculation, ranging from oligemia to extreme hyperemia. The mechanism and the pathogenetic significance of the oligemia cannot be further inferred from the present study. Recently, Ginsberg et al. have reported a similar occurrence of impaired perfusion in a complete global ischemia model in cats.\(^\text{8}\) They have demonstrated that a marked inhomogeneity of blood flow, as well as of energy metabolism, takes place following global ischemia of 30 min.\(^\text{8, 9}\) The pronounced diversity of rCBF response in group A animals in the present experiment is considered to be due to similar inhomogeneous flow in the recirculation period. The results of the present study and Ginsberg's study indicate that post-ischemic inhomogeneous perfusion takes place only when the cerebral tissue has sustained severe damage beyond a certain critical level. Post-ischemic hypoperfusion is more likely the result of brain damage from ischemia rather than the cause of it, but the mechanism of such post-ischemic hypoperfusion remains obscure. The hyperemia during recirculation was strongly associated with the leakage of Evans blue and intracerebral petechial hemorrhages, indicating that hyperemia accelerated the leakage of EB, hence the progression of vasogenic edema. Hyperemia following ischemia has been explained by tissue acidosis and/or dysautoregulation of cerebral vessels.\(^\text{18, 31-33}\) It is still controversial whether post-ischemic hyperemia has a beneficial or detrimental effect on recovery.\(^\text{4, 50, 54}\) The results of this study suggest that the effect of post-ischemic hyperemia is determined by the severity of tissue injury during the ischemia, which, in turn, is determined both by the degree of rCBF reduction and ischemia duration. The critical value of rCBF of 12-15 ml/100 gm/min for 2 hours is in agreement with that of previous reports.\(^\text{35-42}\)

The results of this study suggest that an exact determination of rCBF in ischemic foci is needed for evaluating the indications for surgical revascularization in acute stroke.

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A Tamura, T Asano and K Sano

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