Regional Cerebral Blood Flow and Histopathologic Changes After Middle Cerebral Artery Occlusion in Rats

Hans G. Bolander, MD, PhD, Lennart Persson, MD, PhD, Lars Hillered, MD, PhD, Roland d'Argy, PhD, Urban Ponten, MD, PhD, and Yngve Olsson, MD, PhD

Changes in regional cerebral blood flow were correlated with the distribution of histopathologic signs of brain injury in 35 rats after middle cerebral artery occlusion. Rats were allowed to survive for periods of up to 4 weeks after the operation, and we focused particular interest on the time course of blood flow changes from the initial ischemic events to the late stage of infarction. Regional blood flow was measured using $[14\text{C}]$iodoantipyrine and a quantitative autoradiographic technique. Blood flow in regions with histologic signs of infarction (i.e., the lateral caudoputamen and adjacent neocortex) was below 0.238 ml/g/min, corresponding to 15% of normal values for those regions. In perifocal regions without infarction such as the medial caudoputamen and globus pallidus, cerebral blood flow was also reduced, but it never declined below 20% of its normal value. The decrease in cerebral blood flow was most marked during the first hours after occlusion. Thereafter, cerebral blood flow values gradually normalized, and at 4 weeks there were no significant differences compared with the contralateral side. The border between cortical regions with hypoperfusion and normal cerebral blood flow was rather sharp in the coronal plane, but in the sagittal plane there was a more gradual transitional region. The region with hypoperfusion, observed in the sagittal plane, was most widespread in the acute stage, and normalization of flow occurred particularly from anterior and posterior cortical regions toward the ischemic focus. The possibility for penumbral conditions in the cortex thus exists, particularly in the anterior and posterior borders of the infarction, and remains for several hours after the initial insult. Signs of hyperperfusion were present as an early phenomenon in the globus pallidus and caudoputamen, probably because of altered neuronal activity in line with previous studies or caused by hemodynamic changes in the territory of the occluded middle cerebral artery. Late hyperperfusion was seen in the same regions and also in the neocortex from 18 hours to 7 days after occlusion of the middle cerebral artery. Some of these areas with late hyperperfusion showed histologic signs of definite ischemic changes. The changes in cerebral blood flow occurring in and around a focus of cerebral ischemia have thus been determined in rats with occlusion of the middle cerebral artery. By observing the alterations over a long period of time, we have been able to identify the possibility of penumbral conditions in the early phase and the occurrence of reactive hyperemia in the late stage. (Stroke 1989;20:930-937)
determine the final, permanent changes occurring weeks after the onset of ischemia. Investigations of rCBF have thus far been limited to the acute phase of the ischemic insult. A study of rCBF in relation to histopathologic changes even in the late stages after MCAO is therefore needed. Another reason to expand the observations of rCBF in time is our observation that a substantial restoration of function occurs during at least 6 weeks after MCAO and that the possibility therefore exists that this in some way is reflected in changes in rCBF.

The aim of our study was to investigate rCBF changes over several weeks after MCAO in rats and to correlate these changes with the extent of structural signs of infarction. The data obtained will serve as our baseline for further investigations on therapeutic influences on cerebral infarcts.

Materials and Methods

We used thirty-nine male Sprague-Dawley rats weighing 300–420 g. They were anesthetized for surgical procedures and for blood flow determinations with a mixture of 250 mg/kg body wt chloral hydrate and 50 mg/kg body wt pentobarbital. Rats had free access to food and water before anesthesia. Four rats died and are not included in this study, three of them during preparation for rCBF measurements and one as a consequence of MCAO.

Briefly, our plan was to create focal brain ischemia and infarction in the rats by occluding the MCA and then to allow rats to survive for various periods of time up to 28 days after surgery. The effects of this procedure were investigated in two ways: by observing clinical behavior after occlusion and by determining the character and extent of histopathologic brain changes at the termination of the experiments. Regional cerebral blood flow was examined immediately before the end of the experiments using [%]iodoantipyrine and a quantitative autoradiographic technique. Exposure of the MCA was accomplished with the technique described by Tamura et al. Surgery was performed in an identical way with the exception of coagulation. All rats were breathing spontaneously during this operation except when blood flow determination was made within 1 hour after coagulation. Such rats were intubated and artificially ventilated (see below). Determination of blood flow was made at different time intervals after MCAO: immediately after occlusion (mean 20 min, range 15–30 min), 1 hour, 6 hours, 18 hours, 3 days, 7 days, and 28 days. A separate control group of rats without MCAO had blood flow determination after steady state, and two sham-operated rats were investigated immediately and at 1 hour after steady state.

Neurologic examinations were made before blood flow determinations. The rats were graded into three categories according to Bederson et al with Grade 0=normal behavior, Grade 1=forelimb flexion, Grade 2=forelimb flexion and decreased resistance to lateral push, and Grade 3=same as Grade 2 plus circling behavior.

Frozen histologic sections were taken parallel to those used for blood flow determinations. They were put on glasses in the cryomicrotome and kept in -70°C until their final preparation. The sections were fixed in acetone, stained with Mayer’s hematoxylin and eosin, dehydrated in alcohol and xylol, and mounted. By light microscopy and a camera lucida method it was possible to map out the distribution of infarcts. The information was transferred to sheets of paper to facilitate comparisons with the results from blood flow measurements.

Rats were anesthetized with 70% nitrous oxide and 30% oxygen and artificially ventilated with a small animal respirator. The tail artery and vein and one brachial artery were cannulated for blood sampling, continuous blood pressure recording, infusions, and injections. Rats were paralyzed with 5 mg/kg i.m. suxamethonium chloride and given 50 units heparin. After preparation, rats were allowed a steady-state period to adjust the ventilation to arterial carbon dioxide tension (Paco2) between 4.4–5.5 kPa and oxygen tensions (Pao2) above 12 kPa. Body temperature was kept within normal ranges with a heating system coupled to a rectal thermistor probe.

Regional cerebral blood flow was determined by the [%]iodoantipyrine quantitative autoradiographic technique described by Sakurada et al. Briefly, 45 μCi of 4-iodo-N-methyl [%]iodoantipyrine was infused intravenously over a period of 45 seconds, during which time repeated blood samples were taken from the brachial artery catheter in small glass capillaries. At 45 seconds rats were decapitated, and the brain was rapidly removed from the skull and immersed in isopentane chilled with frozen carbon dioxide to -65°C.

The [%]C activity in the brain was measured by autoradiography. The brain was sliced in 20-μm-thick sections in a cryomicrotome at -20°C. Three sections were taken for autoradiography, two consecutive sections were taken for histologic examination, whereafter 10 sections were discarded and the sequence repeated. The autoradiographic sections were then attached to radiographic film (Kodak SB-5), which was exposed for 1 week, together with a set of calibrated standards.

The optical densities of the autoradiographs were measured in two ways: First, regions of the brain were read manually with a microdensitometer (Joyce-Loebl 3CS, Gateshead, England) then also with a computer-coupled digital image-processing system. The optical densities were measured by the latter system with a video camera (Philips LDH 0400) connected to a computer system (Cromemco 2-HD).
Table 1. Regional Cerebral Blood Flow (ml/min/g) After Middle Cerebral Artery Occlusion in Rats

<table>
<thead>
<tr>
<th>Ipsilateral side</th>
<th>Controls</th>
<th>20 minutes</th>
<th>1 hour</th>
<th>6 hours</th>
<th>18 hours</th>
<th>3 days</th>
<th>7 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory cortex</td>
<td>1.171±.176</td>
<td>.127±.063*</td>
<td>.252±.205*</td>
<td>.503±.264*</td>
<td>.529±.166*</td>
<td>.388±.150*</td>
<td>.449±.107*</td>
<td>.763±.180</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>1.575±.205</td>
<td>.188±.091*</td>
<td>.259±.200*</td>
<td>.514±.263*</td>
<td>.620±.192*</td>
<td>.403±.185*</td>
<td>.569±.083*</td>
<td>.829±.022</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>1.329±.173</td>
<td>.610±.074</td>
<td>1.046±.217</td>
<td>1.198±.158</td>
<td>1.144±.316</td>
<td>1.052±.125</td>
<td>1.026±.310</td>
<td>.945±.331</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>1.552±.200</td>
<td>.276±.137*</td>
<td>.309±.190*</td>
<td>.623±.265*</td>
<td>.733±.166*</td>
<td>.665±.146*</td>
<td>.759±.265</td>
<td>1.044±.015</td>
</tr>
<tr>
<td>Caudoputamen lateral</td>
<td>1.955±.249</td>
<td>.238±.129*</td>
<td>.584±.216</td>
<td>.294±.142*</td>
<td>.451±.221*</td>
<td>.494±.375</td>
<td>.327±.141*</td>
<td>.844±.091</td>
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<tr>
<td>Caudoputamen medial</td>
<td>1.854±.223</td>
<td>.367±.089</td>
<td>.818±.260</td>
<td>1.149±.336</td>
<td>1.095±.390</td>
<td>.867±.280</td>
<td>.798±.029</td>
<td>1.019±.223</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>.930±.104</td>
<td>.647±.211</td>
<td>.545±.068</td>
<td>.791±.037</td>
<td>.888±.197</td>
<td>.914±.154</td>
<td>.932±.120</td>
<td>.674±.158</td>
</tr>
<tr>
<td>Contralateral side</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>1.485±.146</td>
<td>.875±.247</td>
<td>1.056±.191</td>
<td>1.437±.252</td>
<td>1.792±.303</td>
<td>1.209±.072</td>
<td>1.214±.276</td>
<td>.933±.106</td>
</tr>
<tr>
<td>Sensory cortex</td>
<td>1.444±.164</td>
<td>.887±.178</td>
<td>1.007±.185</td>
<td>1.376±.212</td>
<td>1.574±.324</td>
<td>1.139±.062</td>
<td>1.090±.184</td>
<td>.826±.070</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>1.434±.206</td>
<td>.905±.230</td>
<td>1.000±.188</td>
<td>1.415±.212</td>
<td>1.677±.209</td>
<td>1.176±.094</td>
<td>.984±.266</td>
<td>.844±.132</td>
</tr>
<tr>
<td>Cingulate cortex</td>
<td>1.307±.159</td>
<td>.722±.220</td>
<td>.806±.168</td>
<td>1.365±.376</td>
<td>1.356±.254</td>
<td>1.018±.124</td>
<td>.819±.221</td>
<td>.842±.151</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>1.434±.231</td>
<td>.953±.257</td>
<td>1.004±.158</td>
<td>1.274±.113</td>
<td>1.821±.388</td>
<td>1.094±.080</td>
<td>1.198±.373</td>
<td>.743±.028</td>
</tr>
<tr>
<td>Caudoputamen lateral</td>
<td>1.739±.171</td>
<td>1.014±.182</td>
<td>1.070±.169</td>
<td>1.738±.357</td>
<td>2.095±.281</td>
<td>1.156±.160</td>
<td>1.170±.142</td>
<td>1.146±.156</td>
</tr>
<tr>
<td>Caudoputamen medial</td>
<td>1.711±.252</td>
<td>.967±.289</td>
<td>1.000±.137</td>
<td>1.755±.479</td>
<td>1.932±.335</td>
<td>1.085±.146</td>
<td>.982±.133</td>
<td>1.157±.153</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>.864±.079</td>
<td>.630±.171</td>
<td>.654±.074</td>
<td>.767±.175</td>
<td>1.112±.201</td>
<td>.654±.053</td>
<td>.610±.165</td>
<td>.487±.009</td>
</tr>
</tbody>
</table>

Values are mean±SEM.
*Significantly different from contralateral side, p<0.10, when tested with t test.

via an image digitizer (Cromemco SDD). The digitizer provides 256 different gray levels and 384×241 "unit areas" (pixels). The unit area of measurement selected was approximately 70 μm².

In both types of measurements blood flow values were calculated from the optical densities, arterial ¹⁴C activity curve, using a blood–brain partition coefficient of 0.8 and the equation described for the method by Sakurada et al.⁹ The microdensitometer measures optical densities in different anatomic regions based on the average of several measuring points within this region. The averages of these values for all rats are given in Table 1. In the process of evaluating the autoradiographs for hypoperfusion and hyperperfusion, visual inspection of these and the computer-made images were made since such images better demonstrate flow variations within a region. If there were both hypoperfusion and hyperperfusion within the same anatomic region, repeated measurements were made with the densitometer. An area was noted as hypoperfused if the value was below 50% of the contralateral side in a substantial part (>50%) of the anatomic region and hyperperfused if the value was more than 30% above the value on the contralateral side (i.e., 130%). Since these areas sometimes crossed the borders for our anatomic regions, the areas were classified into the region where most of the area was situated if several anatomic areas were not substantially involved. This means that an area can be noted as both hypoperfused and hyperperfused if, for example, an infarct is surrounded with a small rim of hyperperfusion.

The definitions of the anatomic regions are as follows: Frontal cortex, in the dorsal plateau of the cortex rostral to caudate; sensorimotor cortex, in sections rostral to the largest diameter of the caudate; parietal cortex, in the dorsal plateau of the cortex in sections first displaying caudate-putamen (going in rostral direction); cingulate cortex, cortex near the interhemispheric fissure at the level of the caudate; auditory cortex, at the level of the medial geniculate; caudoputamen lateral, lateral and anterior half of the caudate; caudoputamen medial, medial and posterior half of caudate; globus pallidus, in sections where caudate is crescent in shape.

Results

Table 2 shows the physiologic variables for rats in the control and in different experiment groups. There were no significant differences between control and experimental groups except for a lower Pco₂ in rats measured 20 minutes after MCAO.

Neurologic examination could not be performed until 6 hours after MCAO since rats were not yet fully awake. Improvement of neurologic function was observed after 1 week in accordance with our previous results.⁸ The majority of rats were scored as Grade 2 during the 1st week (Table 2).

In the histologic sections from rats killed within 1 hour of ischemia, only small abnormal areas were present in the basal part of sensorimotor cortex near the site of craniectomy. Corresponding auto-
radiographs showed extensive areas of hypoperfusion in the caudoputamen and neocortex (Table 3), that is, in a much larger area of the brain. Most likely, based on frozen sections of unfixed tissue, the structural changes were too subtle to be assessed with the histologic technique applied. It is a well-known fact that very early ischemic changes require optimal processing techniques including fixation by vascular perfusion.

In the group of rats killed between 6 hours and 3 days, it was easy to delineate the infarcted area. Altered tissue was present in the caudoputamen and surrounding neocortex, but not in the medial parts of caudoputamen (Table 3). In the neocortex, infarcted area was seen as far back as the parietal cortex, and only one rat had ischemic changes dorsal to it. The most typical pattern, found in eight out of 12 rats, was an infarct that occupied both caudoputamen and neocortex. Two rats had infarcts confined to the neocortex or caudoputamen.

In the groups killed after 1 week, the infarct was well demarcated. In those killed at 4 weeks, there was a reduction in size of the infarcted hemisphere as previously described.8

Table 1 gives the CBF values for ipsilateral and contralateral sides of the neocortex, caudoputamen, and globus pallidus in the different groups of rats, killed at time intervals from 20 minutes to 4 weeks after MCAO. Figure 1, top and bottom, gives a graphic illustration of the relative CBF values in percentages of normal values for each localization in the ipsilateral side.

Structures with the highest frequency of infarction (i.e., lateral caudoputamen and frontal, sensorimotor, and parietal cortex) initially have values below 15% of their normal values corresponding to a flow of 0.127–0.238 ml/min/g tissue in absolute values. The groups killed at 6 hours to 3 days after MCAO showed an increase to 20–40% of normal values in the same regions. However, if rats without infarction are excluded (broken lines in Figure 1), the level of CBF is still below 20% at 6 hours. In the medial caudoputamen and auditory cortex, where few or no rats develop infarcts, the initial CBF values were 20% and 19%, respectively, corresponding to 0.367 and 0.276 ml/min/g tissue in absolute values. It therefore seems likely that a threshold for
ischemic damage is between 15% and 20% of normal flow.

In general, there are low values in the early stages and then a slow trend toward more normal values. At 4 weeks there were no significant differences from the contralateral side (Table 1).

There were no significant differences between ipsilateral and contralateral side values in control rats. In the sham-operated rats, flow was reduced on the ipsilateral side in the two regions adjacent to the craniectomy, that is, the sensorimotor and parietal cortex and lateral caudoputamen. The flow in these two areas was reduced to 66% and 73%, respectively, of the value on the contralateral side.

Table 3 shows the distribution of hypoperfusion and hyperperfusion in different anatomic regions. Areas of hypoperfusion were relatively evenly distributed between the groups up to 1 week after MCAO. An exception was found in the lateral caudoputamen of the group killed 1 hour after MCAO, where only one rat showed signs of hypoperfusion. The distribution of structural changes (the numbers in parentheses) in rats killed 6 hours to 3 days after MCAO corresponds to the regions showing hypoperfusion in the MCA territory, that is, the sensorimotor and parietal cortex and lateral caudoputamen. However, in more peripheral parts of the lesion, hypoperfusion is not always associated with detectable structural changes.

A close correlation between the area of hypoperfusion and the area of infarction could also be seen when camera lucida drawings from the histologic sections outlining the infarcted area were superimposed on the digitized and color-coded pictures of the autoradiographs (Figures 2 and 3). The area of hypoperfusion has a distinct border with a very narrow transitional region between low and more normal flows in this coronal plane. Indeed, there is a 10-fold increase in flow within less than 1-mm distance. However, looking at neocortical flow longitudinally by measuring the same sector of cortex in successive autoradiographic sections, 0.6 mm apart, the same transition is not so sharp (Figure 4).

To avoid the effects of surgical trauma in the lower part of the cortex and the collateral flow from the anterior cerebral artery coming from the medial upper part, the sector we chose to measure was the middle third of the cortex in the coronal plane. The middle sector probably has most of its collateral flow from the anterior and posterior cerebral circulation.

Figure 4 also illustrates that CBF is lowest in the acute stage and then increases. The rate of improvement is greater and more marked in the anterior and posterior peripheral parts of cortex from where collateral flow is emerging. The transition from lowest to normal flow is gradual.

Hyperperfusion areas were seen in the globus pallidus and caudoputamen both early and late after MCAO (Table 3). The distribution of hyperperfusion seems to have two maxima: early hyperperfusion in the acute stage up to 6 hours after occlusion in the globus pallidus and the caudoputamen. Late hyperperfusion from 18 hours to 7 days was seen also in neocortical structures. Hyperperfusion did not correlate with histologic changes. No changes were found in the acute stage. Three out of four rats killed 3 days after MCAO had collections of polymorphonuclear leukocytes and macrophages in the hyperperfused areas. Also in three out of seven rats with late hyperperfusion these areas showed histologic signs of definite ischemic damage (Figure 2).

Discussion

Our study confirms that this model is a reliable tool for the study of focal cerebral ischemia. The distribution of hyperperfusion areas and infarcted brain tissue is similar to that described by several other authors with this model.1-3,5,6 The model carries very low mortality, and rats develop neuro-
logic deficits, the severity of which is easily scored into different categories.4 However, long-term follow-up of this neurologic deficit shows that a considerable restitution appears more than 7 days after MCAO.8

The initial rCBF value in regions with infarcted tissue was lower than 0.238 ml/min/g, and in peri-infarct regions with normal histologic appearance initial rCBF was above 0.276 ml/min/g. This coincides with other observations using the same species and technique of CBF measurement.2,3 In other species, lower flows were found in the infarct and peri-infarct region with the hydrogen clearance technique.10-11 The blood flow values were lowest in the acute stage, and they tended to normalize over time as previously described by Nedergaard et al.5

The hypoperfused areas occupied larger regions of neocortex than did the infarcted tissue seen by microscopy. Looking at coronal sections, the border between ischemia and normal flow was rather sharp. In our reconstruction of neocortical flows in the sagittal plane (Figure 4), a less distinct border is seen. Furthermore, in the acute stage the area of hypoperfusion was larger, and the transition between low and more normal flows was more gradual.

This means that some of the cortical areas could be in "penumbral conditions," that is, areas with functional failure due to hypoperfusion but with potential for recovery when the blood flow is normalized. It is also clear from our study that normalization of flow starts in the frontal and occipital regions of the affected tissue where collateral flow from anterior and posterior cerebral arteries are coming from.12 The importance of this collateral circulation has been emphasized in earlier reports.13,14 Also, pretreatment with nimodipine decreases the area of infarction not in the center of infarction but at the borders.15

The lowest rCBF was found in the acute stage after MCAO, but values remained <20% of normal up to 6 hours after the vascular occlusion. In border zones of infarction (medial part of caudoputamen and auditory cortex), flow was never <20% of normal and increased earlier. The possibility for "penumbral conditions" thus seems to exist for several hours, and therapeutic interventions during this time could be expected to alter the severity of the resulting infarction. The fact that nimodipine, a calcium-antagonist, has been found effective in diminishing infarct size in this model if given earlier than 6 hours after MCAO supports such a theory.16

Otherwise, the general pattern is that of a slow return to normal values in all regions as reparative processes proceed. After 4 weeks the measured values are equal to those on the contralateral side. This is in agreement with the findings of Yamaguchi et al17 in a study on focal ischemia in cats.

Areas of hyperperfusion were seen in our material both in the acute stage after MCAO and later, as also described by Heiss et al.18 In the acute stage the hyperperfusion zones were located in the rim of the hypoperfusion in the caudoputamen or in the globus pallidus. Such early hyperperfusion areas have been reported previously in rats.2,19

We do not know if all areas of hyperperfusion are located outside or within the area to be infarcted.
However, globus pallidus showing hyperperfusion never developed an infarct. It has previously been suggested that hyperperfusion might be due to tissue acidosis or "luxury perfusion."20 This seems unlikely in the present experiments since a tissue acidosis should spread more evenly around the infarcted area, and hyperperfusion would then also be seen in cortical structures. One possible explanation is a neuronal or metabolic hyperactivity as suggested by Tamura et al,2 also coupled to an increased glucose use in the same areas.5,19 Another possibility is a redistribution of flow in the MCA after occlusion. Since the occlusion is made proximal to the lateral lenticulostriate group supplying the lateral part of the caudoputamen but distal to the medial lenticulostriate group supplying the medial and posterior part of caudoputamen,12 an occlusion might induce an increase of flow in the medial parts of the caudoputamen and globus pallidus.

Eighteen hours after MCAO, hyperperfusion was also seen in cortical structures but even more frequently in the caudoputamen and globus pallidus. Some hyperperfusion was seen inside infarcted area as also reported by Yamaguchi et al.17 Three days after MCAO, the infarct is in transition between maturation and resolution.8 Infiltration of leukocytes was also seen in connection with these late hyperperfusion areas in this material. This points to a mechanism of reactive hyperemia or neovascularization as the cause of this hyperperfusion.

In conclusion, our study shows that penumbral conditions may exist in the border zone around cortical brain infarct after MCAO in rats, which makes rats suitable for the study of pharmacologic treatment of focal cerebral ischemia. With this long-term study of CBF after MCAO, we have been able to identify patterns of hyperperfusion that occur early and late after the insult and probably on the basis of different mechanisms. We will use these
data as a basis in our future research on prevention of tissue damage in focal cerebral ischemia.

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References

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