Hemorrhagic Infarct Induced by Arterial Hypertension in Cat Brain Following Middle Cerebral Artery Occlusion

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The purpose of this experiment was to determine whether an acute rise in brain perfusion pressure causes hemorrhagic transformation of an infarct without a reopening of the occluded artery. We raised the blood pressure of 22 cats by aortic obstruction 5-24 hours after transorbital middle cerebral artery clipping; hemorrhagic infarcts were induced in 11. Mean arterial blood pressure increased by 57.2 ± 16.9 mm Hg (mean ± SD) in the 11 cats with hemorrhagic infarcts and by 40.4 ± 16.9 mm Hg in the 11 remaining cats with pale brain infarcts (p < 0.05). Induction of hypertension increased regional cerebral blood flow in the ischemic cortical gray matter more in three cats with hemorrhagic infarcts than in seven with pale infarcts. Our results demonstrate that hemorrhagic transformation of an infarct can be induced by a rapid increase in perfusion pressure to brain tissue already exposed to focal ischemia. We also suggest that the restoration of blood flow through leptomeningeal collaterals plays an important role in the pathogenesis of hemorrhagic infarction in cases without reopening of occluded arteries. (Stroke 1990;21:589-595)

Since Fisher and Adams first reported autopsy cases of cerebral embolism, hemorrhagic brain infarction has been thought of as a consequence of reperfusion of the damaged vascular bed by a reopening of the occluded artery. However, we recently demonstrated in human autopsy cases that hemorrhagic transformation of an infarct in persons with cardioembolic stroke may occur without a reopening of the occluded artery and that restoration of blood flow to the infarcted area through leptomeningeal collaterals on the surface of the brain is likely to be responsible for the hemorrhagic transformation in cases with surges of arterial hypertension.

The pathogenesis of hemorrhagic infarction has been studied in experimental animals with and without reopening of the occluded artery. In the latter, hemorrhagic transformation was attributed to underlying pathologic changes of the vasculature, such as hypertensive vascular changes preceding ischemia or degenerative or proliferative vascular changes secondary to ischemia. These factors operate in the presence of sufficient perfusion pressure of the infarcted area through leptomeningeal anastomoses. However, no previous study evaluated the mechanisms we proposed based on our observations of human autopsy cases. Also, especially in early reports, physiologic parameters were not adequately monitored. Thus, we designed this experiment to clarify whether a rapid rise in blood pressure causes hemorrhagic transformation of an infarct without a reopening of the occluded artery and to assess the relation between the pathologic distribution of ischemic changes and hemorrhage and the regional cerebral blood flow (rCBF) in cats with induced hypertension after middle cerebral artery (MCA) occlusion.

Materials and Methods

We anesthetized 29 cats weighing 2.4-4.3 kg with 10 mg/kg i.m. ketamine hydrochloride. A double-lumen balloon catheter (Swan-Ganz, American Edwards Laboratories, Irvine, California) was inserted through the femoral artery. The tip of the catheter was placed in the descending thoracic aorta immediately distal to the left subclavian artery for measurement of blood pressure, sampling of blood, and induction of hypertension by inflating the balloon. Aortic blood pressure was measured with a Statham P23 ID pressure transducer (Gould Inc., Cleveland, Ohio). A femoral vein was cannulated for...
After tracheostomy, each cat was immobilized with 10 mg/kg i.v. decamethonium bromide and mechanically ventilated with 70% N₂O and 30% O₂. The end-tidal CO₂ concentration was continuously monitored with an infrared CO₂ analyzer (Capnometer, Hewlett-Packard, Palo Alto, California) and maintained at approximately 33 mm Hg throughout the experiment by adjusting the respiratory rate and volume. The cat's head was placed in a David Kopf stereotactic frame (Tujunga, California). The left MCA was exposed transorbitally with the aid of an operating microscope, and focal cerebral ischemia was induced by clipping the MCA with a Zen's clip (a temporary clip for microsurgery) at its origin (where it branched from the internal carotid artery) for 5–24 hours. Arterial blood was periodically sampled for the determination of Paco₂, Paco₂, and pH using an acid–base analyzer (ABL30, Radiometer, Copenhagen, Denmark). Blood gases and pH were maintained within physiologic ranges. Body temperature was maintained at 37–38°C using a homeo-static heat blanket servo-controlled by a rectal temperature probe.

Blood pressure was raised in 24 cats by aortic obstruction (inflating the balloon catheter) for the last hour of focal cerebral ischemia. Brains from two of the 24 cats were excluded from the study since ischemic changes were observed only in the basal ganglia on gross as well as microscopic examination. Blood pressure was not raised in the five remaining (control) cats, which were killed 5, 7, 11, 16, and 24 hours after MCA occlusion.

In 12 of the 22 cats with aortic obstruction, rCBF was measured by the hydrogen clearance method before and 30 minutes after MCA occlusion and 30 minutes before and 30 minutes after aortic obstruction. Burr holes 2 mm in diameter were made using an air drill, and the dura was removed. Teflon-coated platinum electrodes 200 µm in diameter were stereotactically placed in the gray matter of the ectosylvian gyrus and in the subjacent deep white matter of both cerebral hemispheres with reference to a stereotactic atlas of the cat brain. The burr holes were closed with dental cement. rCBF was determined from the initial slope method following the administration of 10–20% H₂ in the inspired gas. CBF values obtained from improperly placed electrodes were excluded from the study.

In all 27 cats, the blood–brain barrier was evaluated using 2 ml/kg i.v. 5% Evans blue 15 minutes before perfusion of the brain to confirm the positions of the electrodes relative to the infarct. The cats were killed by transcardiac perfusion with 4.0% formaldehyde buffered to a pH of 7.3 with 0.07 M sodium phosphate for 30 minutes at the MABP of each animal before aortic obstruction; the left MCA was still clipped. The skull was opened, and the brain was immersed in the buffered formaldehyde solution for 1 week. The position of the clip occluding the MCA was confirmed. Coronal slices taken at approximately
3-mm intervals were dehydrated and embedded in paraffin; 5-μm sections were stained with hematoxylin and eosin and other methods as needed for histologic examination.

All data are expressed as mean±SD. Differences between groups were evaluated using Student’s t test. The 2×2 tables (ischemia lasting ≤12 vs. >12 hours, MABP increase of <40 vs. ≥40 mm Hg) were analyzed using Yates’ corrected χ² test. If the expected number was ≤3, the 2×2 tables were analyzed using Fisher’s exact probability test.

An additional experiment was performed to confirm the ability of the Zen’s clip to occlude an artery during induced hypertension. One common carotid artery of an adult Wistar rat was exposed under anesthesia with 50 mg/kg i.p. pentobarbital and occluded with the clip. The artery distal to the clip was transected after ligating the distal portion. The surgical field was filled with saline. The stump of the transected artery was observed with an operating microscope for leakage of arterial blood during hypertension induced by temporary clipping of the abdominal aorta and by a bolus injection of 10 μg/kg i.v. norepinephrine.

Results

The left cerebral hemisphere of all 27 cats showed pathologic evidence of cerebral infarction in the area supplied by the occluded MCA. Of the 22 cats with aortic obstruction, 11 showed hemorrhagic infarcts (three were macroscopic and the other eight were microscopic). Brains from the other 11 cats did not show any evidence of hemorrhage in the infarct. There was no hemorrhage in the brains of any of the five control cats.
TABLE 1. Physiologic Parameters in Cats Before and After Middle Cerebral Artery Occlusion and Aortic Obstruction

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before occlusion</th>
<th>30 minutes before obstruction</th>
<th>30 minutes after obstruction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemorrhagic infarct (n=11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PacO₂ (mm Hg)</td>
<td>34.6±6.3</td>
<td>33.1±8.6</td>
<td>33.2±6.0</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>150.8±23.8</td>
<td>148.3±25.5</td>
<td>148.2±17.6</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.7±3.1</td>
<td>38.9±4.3</td>
<td>38.0±4.4</td>
</tr>
<tr>
<td>pH</td>
<td>7.32±0.07</td>
<td>7.35±0.05</td>
<td>7.34±0.05</td>
</tr>
<tr>
<td><strong>Pale infarct (n=11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PacO₂ (mm Hg)</td>
<td>33.5±5.7</td>
<td>32.4±5.9</td>
<td>32.4±5.8</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>153.2±22.9</td>
<td>149.4±23.7</td>
<td>151.7±21.9</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.3±3.2</td>
<td>37.9±2.7</td>
<td>37.4±2.5</td>
</tr>
<tr>
<td>pH</td>
<td>7.34±0.07</td>
<td>7.34±0.09</td>
<td>7.35±0.06</td>
</tr>
<tr>
<td><strong>Controls (n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PacO₂ (mm Hg)</td>
<td>30.4±7.4</td>
<td>33.2±7.2</td>
<td>—</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>142.6±26.4</td>
<td>154.6±20.8</td>
<td>—</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>38.3±3.3</td>
<td>37.3±3.5</td>
<td>—</td>
</tr>
<tr>
<td>pH</td>
<td>7.32±0.07</td>
<td>7.33±0.04</td>
<td>—</td>
</tr>
</tbody>
</table>

Values are mean±SD.

Gross pathologic examination of the brains showed swelling of the left cerebral hemisphere in all 22 cats with aortic obstruction; there was no herniation. On coronal sections, there was softening of the basal ganglia, the overlying cerebral cortex including the ectosylvian gyrus, and the subjacent white matter on the left side. The cortical gray matter and basal ganglia of the softened area were stained with Evans blue (except in three cats with MCA occlusion for 5–7 hours). The white matter was not stained in any cat. A large part of the cortical gray matter and basal ganglia in the softened area was speckled with many hemorrhagic spots in three cats. The hemorrhages were more obvious after extraction of Evans blue by 70–80% alcohol during processing of the microscopic sections (Figure 1).

Brain sections of the 22 cats with aortic obstruction stained with hematoxylin and eosin showed diffuse pallor and sponginess of the caudate nucleus, putamen, claustrum, the anterior and middle portion of the internal capsule, and the overlying cortex and subjacent white matter of the frontotemporalparietal region on the left side, which are supplied by the occluded MCA (Figures 1 and 2).13,15 The cortical gray matter and basal ganglia showed ischemic changes such as shrinkage and eosinophilia of the cytoplasm, smudgy deep purplish staining of the nuclei of the neurons, enlarged perineuronal spaces, and vacuolation of the neuropil. The white matter was vacuolated. The blood vessels within the infarct showed no proliferative changes. Three brains with macroscopic hemorrhagic spots in the infarct showed many erythrocytes extravasated from the small vessels and capillaries (Figure 2); eight additional brains showed occasional microscopic hemorrhagic spots scattered throughout the infarcted cerebral cortex and basal ganglia not visible macroscopically. The other 11 brains contained neither macroscopic nor microscopic hemorrhages. No hemorrhages were observed in the deep white matter of the ischemic hemisphere or in the gray and white matter of the nonischemic cerebral hemisphere in any brain.

The five control cats showed similar ischemic changes on gross and microscopic examination but did not show any hemorrhagic spots in the infarct. PaCO₂, PaO₂, arterial pH, and hematocrit were maintained within normal ranges and did not change signif-

TABLE 2. Mean Arterial Blood Pressure in Cats Before and After Middle Cerebral Artery Occlusion and Increase Induced by Aortic Obstruction

<table>
<thead>
<tr>
<th>Group</th>
<th>Before occlusion</th>
<th>30 minutes after occlusion</th>
<th>Increase at 30 minutes after occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemorrhagic infarct (11)</td>
<td>139.4±10.8</td>
<td>140.2±14.8</td>
<td>138.8±15.3</td>
</tr>
<tr>
<td>Pale infarct (11)</td>
<td>143.2±9.1</td>
<td>138.2±11.7</td>
<td>133.7±17.8</td>
</tr>
<tr>
<td>Control (5)</td>
<td>130.6±15.7</td>
<td>130.8±10.7</td>
<td>136.6±9.0</td>
</tr>
</tbody>
</table>

Values are mean±SD. Increase, difference in values 30 minutes before and 30 minutes after aortic occlusion. *p<0.05 different from pale infarct by Student's t test.
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4 6 8 10 12 14 Duration of Ischemia (hours)

20 22 24 16 18

FIGURE 3. Scatterplot of increase in mean arterial blood pressure (MABP) induced by aortic obstruction and duration of focal cerebral ischemia in cats by pathologic findings of infarct: ■, Macroscopic hemorrhage; ○, Microscopic hemorrhage; ◦, pale infarct.

significantly during the experiment. The values did not differ significantly among the three groups (Table 1).

Before aortic obstruction, MABP did not change significantly during the experiment and did not differ among the three groups (Table 2). Aortic obstruction resulted in a prompt and sustained increase in MABP ranging from 10 to 93 mm Hg. The increase was significantly greater in cats with hemorrhagic infarcts than in cats with pale infarcts (57.2±16.9 vs. 40.4±16.9 mm Hg, p<0.05).

Figure 3 shows the increase in MABP and the duration of ischemia for the 22 cats with reference to the pathologic findings of their infarcts. The rate of hemorrhagic transformation was significantly greater in cats with an MABP increase of >40 mm Hg than in cats with an MABP increase of <40 mm Hg (75% vs. 20%, p<0.05). Also, in cats with cerebral ischemia for >12 hours, the rate of hemorrhagic transformation was significantly greater than in cats with cerebral ischemia for ≤12 hours (64% vs. 36%, p<0.05).

Of the 12 cats with rCBF measurement, three showed microscopic hemorrhagic infarcts and the other nine showed pale infarcts. rCBF of the 12 cats before MCA occlusion was 67.2±15.1 (n=10) and 26.6±7.7 (n=11) ml/100 g/min in the ectosylvian gyrus and the subjacent deep white matter on the left side and 63.8±14.6 (n=8) and 23.6±8.1 (n=10) ml/100 g/min, respectively, on the right side. Thirty minutes after MCA occlusion, rCBF decreased to 10.2±5.8 and 7.3±3.3 ml/100 g/min in the ectosylvian gyrus and the subjacent deep white matter on the left side, while it was 55.0±11.0 and 19.7±7.4 ml/100 g/min, respectively, on the right side. Thirty minutes before aortic obstruction, rCBF was 12.4±7.8 and 5.9±3.1 ml/100 g/min in the ectosylvian gyrus and the subjacent deep white matter on the left side.

Figure 4 shows the relation between the increase in MABP and the increase in rCBF in the cortical gray matter of the ectosylvian gyrus and the subjacent deep white matter on both sides in each cat. When MABP was raised rCBF increased on both sides. The increase in rCBF in the cortical gray matter was more prominent in the nonischemic right side than in the ischemic left side. rCBF in the cortical gray matter of the left ectosylvian gyrus increased significantly more in three cats with hemorrhagic infarcts than in seven cats with pale infarcts (16.3±6.9 vs. 4.6±2.3 ml/100 g/min, p<0.05). rCBF in the deep white matter increased slightly on both sides, but this increase was much less than that in the cortical gray matter.

Left cortical gray matter

Right cortical gray matter

Left deep white matter

Right deep white matter

FIGURE 4. Graph of relation between increase in regional cerebral blood flow (rCBF) and increase in mean arterial blood pressure (MABP) induced by aortic obstruction in cortical gray matter of ectosylvian gyrus and subjacent deep white matter of ischemic left and nonischemic right cerebral hemispheres of cats. Each spot refers to value in designated area. Values from improperly placed electrodes are excluded. ●, Hemorrhagic infarct in corresponding site.
The experiment to confirm the ability of the Zen's clip to occlude an artery revealed that no blood leaked from the stump of the common carotid artery when MABP was raised from 100 to 150 mm Hg by clamping the abdominal aorta and to 190 mm Hg by injecting norepinephrine.

**Discussion**

Hemorrhagic transformation of a cerebral infarct through a rapid increase in blood pressure was induced without a reopening of the occluded artery in this experimental model. Since the Zen's clip on the MCA did not allow leakage of blood during a rapid and sustained rise in blood pressure (as shown in the additional experiment in a rat), the source of blood flow to the infarcted area is considered to be the leptomeningeal collaterals on the surface of the brain in retrograde fashion. These collateral channels have been demonstrated in humans as well as in cats and dogs.

Hemorrhages into infarcted areas have been investigated using various animal models. There have been many reports of reperfusion via a reopened arterial occlusion. In these reports, the hemorrhagic infarct could not be induced unless vascular wall damage was sufficient to allow leakage of intravascular components.

Several models induce hemorrhages in infarcts of animals with permanent arterial occlusions. Hypertension induced by neosynephrine and aortic clamping 7 days after MCA occlusion resulted in massive cerebral hemorrhage in the infarcted area. Decreased tissue resistance near a degenerating brain blood vessel was regarded to be an essential factor in inducing the cerebral hemorrhage. Faris et al produced hemorrhagic infarcts by ligating the MCA for 2–14 days in dogs with previous hypertension for 2–5 weeks. Systolic blood pressure was increased 40–70 mm Hg by surgical coarctation of the aorta. Systemic hypertension, hypertensive arteriolar changes in the brain preceding focal ischemia, and patent leptomeningeal anastomoses were regarded as being the factors necessary to produce hemorrhagic infarction. Laurent et al induced acute hypertension by norepinephrine infusion 24 hours after occluding a MCA in rhesus monkeys; this caused a larger infarct than that in normotensive monkeys. The authors stated that acute hypertension 5 days after MCA occlusion resulted in hemorrhagic transformation of the infarct and that hypercarbia 5 days after MCA occlusion resulted in intracerebral hematoma formation. The authors emphasized the importance of vascular regenerative changes and vasodilatation in the pathogenesis of hemorrhagic infarction.

We raised arterial blood pressure 5–24 hours after MCA occlusion so that no blood vessels underwent proliferative or regenerative changes pathologically. Thus, our experimental model is most suitable for the investigation of the pathogenesis of hemorrhagic infarction without a reopening of the occluded artery, which has been confirmed in human autopsy cases of cardioembolic stroke. The incidence of hemorrhagic brain infarction increased in cats with an MABP increase of ≥40 mm Hg and in cats with focal cerebral ischemia for >12 hours. When MABP was raised, rCBF in the cortical gray matter increased on both sides of the brain, and in particular, rCBF in the ischemic cortical gray matter increased significantly more in cats with hemorrhagic infarcts than that in cats with pale infarcts. These results indicate that a rapid increase in perfusion pressure to the ischemic brain leads to an increase in rCBF to the gray matter, which becomes the prerequisite for hemorrhagic transformation of an infarct.

We also demonstrated the difference between the increase in rCBF in the cortical gray matter and that in the deep white matter in response to a rise in perfusion pressure to brain tissue that has been exposed to focal ischemia. When MABP was raised, rCBF in the deep white matter increased much less than that in the cortical gray matter on both sides of the brain. This observation is compatible with the pathomorphology of hemorrhagic infarction, that is, cortical gray matter becomes hemorrhagic while the deep white matter remains pale. The absence of extravasated blood in the deep white matter can be explained by its characteristic vascular architecture. The deep white matter is poorly vascularized, supplied by longer and less branched penetrating vessels that would be readily compressed by the increased tissue pressure caused by edema. On the contrary, the cortical gray matter is supplied by short penetrating branches from the leptomeningeal arteries that end in the cortical gray matter with short branches to dense capillary networks. Therefore, the cortical vessels that lapsed in vasoparalysis are likely to be exposed to the full force of arterial blood pressure and become foci of bleeding when hemorrhagic transformation takes place.

In conclusion, we verified that hemorrhagic transformation of an infarct caused by permanent occlusion of the proximal artery can be induced by an increase in perfusion pressure to the infarcted area, probably through leptomeningeal collaterals in a retrograde fashion. These results imply that a hemorrhagic infarct in a person with cardioembolic stroke is not always caused by a reopening of the occluded artery and that structural damage to the brain would be worsened by hemorrhages within the infarct caused by excessive elevation of blood pressure even in persons with persistent occlusion of the proximal artery.

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**References**


KEY WORDS • cerebral blood flow • cerebral hemorrhage • hypertension • cats
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