Cerebral Circulation After Cardiac Arrest
Microangiographic and Protein Tracer Studies

SHU-REN LIN, M.D.*, and MARTTI KORMANO, M.D.†

SUMMARY The effects of ventricular fibrillation and subsequent resuscitation on the microcirculation of the cerebral cortex were studied with microangiographic and fluorescent protein tracer techniques. Immediately after revival, a transient period of impaired cerebral perfusion occurred before complete recovery from circulatory arrest was obtained. A circulatory arrest of longer than ten minutes, followed by four to six hours of resuscitation, caused defects of cortical capillary filling in both microangiography and Trypan blue fluorescence. This was considered to represent impairment of cortical perfusion, most likely due to edema. Cardiac arrest up to 12 minutes and subsequent resuscitation per se caused no blood-brain barrier damage. Minimal blood-brain barrier damage occurred in one dog following uncomplicated revival from a 14-minute arrest and in animals with prolonged hypertension postresuscitation. Up to three consecutive carotid angiographies did not cause blood-brain barrier damage in the postischemic brain.

Introduction

THE INABILITY of a patient to recover from cardiac arrest in spite of successful resuscitation is most likely secondary to anoxic damage of the central nervous system. The ultimate degree of brain damage sustained after cerebral ischemic-anoxic insult is not entirely due to initial insult but also due to some postischemic pathophysiological changes which may be amenable to therapy.1 When cardiac arrest in a dog lasts longer than five minutes, deterioration of the brain circulation of a successfully resuscitated animal will occur three to four hours later, in the absence of changes in general blood pressure, EKG or arterial blood gas changes.2-4 Carbon black perfusion studies show localized defects of perfusion which may be caused by a combination of vascular and parenchymal changes.4 Arterial vessels that regulate the supply of blood to central nervous system tissue are at most 50 to 70 μ in diameter or even smaller.6 Such vessels cannot be demonstrated by cerebral angiography, but are readily visualized by microangiography. Intravascular administration of Trypan blue produces red fluorescence of perfused blood vessels in tissue sections examined by fluorescence microscopy. Major defects of perfusion and blood-brain barrier (BBB) damage are demonstrated as lack of fluorescent capillary cross sections and extravasation of the red fluorescence, respectively.6 Since it is likely that the late progressive deterioration of the cerebral circulation in cardiac-arrested dogs occurs in the microvascular level, a microangiographic and Trypan blue fluorescence study of cardiac-arrested dog brain was carried out.

Methods

Twenty-four male mongrel dogs weighing approximately 20 kg were used in this experiment. The animals were anesthetized with intravenous sodium pentobarbital (25 mg per kilogram). An endotracheal tube was placed and connected to a Harvard respirator. Each animal received a mixture of 50% oxygen and 50% nitrous oxide. The respiratory rate was adjusted to maintain PCO2 within normal range. Rectal temperature was maintained at 37°C. The femoral arteries and veins were exposed and connected to catheters for recording of blood pressure and venous pressure and for withdrawal of blood samples for blood gas analysis. Blood samples were taken from each animal for arterial blood gas analysis before and every hour after restoration of blood flow. Constant intravenous fluid (0.9% NaCl) was slowly given to each animal. Urine was collected every hour for creatinine clearance determination.

Cardiac arrest was produced by fibrillating the heart (1 to 2 volts using an AC fibrillator) after opening the chest. Heart beat was restored to normal after 5 to 15 minutes of cardiac arrest by defibrillation (20 to 40 joules using a DC defibrillator) alone or combined with massage and occasional direct injection of epinephrine into the heart. Failure of revival occurred in one animal (not included in the series). Blood gases and blood pH were adjusted to normal as fast as possible by intravenous infusion of bicarbonate, by adjustment of respiratory rate and by addition of oxygen to the inspired air. Each animal was then followed for up to six hours after restoration of normal blood flow.

The design of experiment among 12 dogs used for microangiographic study is presented in table 1. At the end of the experimental procedure 1,000 ml of a 15% saline suspension of barium sulfate (Micropaque, Damancy & Co.) at 37°C were introduced into the ascending aorta through a femoral catheter. The first infusion was followed by 1,000 ml of 15% Micropaque suspended in buffered 4% formaldehyde. The infusion pressure was maintained slightly higher than the animal’s systolic pressure. The catheters at the femoral veins were opened to avoid excessive overloading of the vascular bed. Cardiac function continued up to the end of the infusion of the saline suspension. The degree of capillary filling was inadvertently compromised by introducing the dye into the ascending aorta instead of the carotid, in order to provide a more physiological distribution of the dye during the infusion of the anesthetized animal. It was also possible to maintain the normal blood pressure until the animal died when the formaldehyde suspension was infused.

After completion of the Micropaque infusion the brain was removed, fixed in formaldehyde and cut into 1-mm and
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### Table 1 Microangiography of Dog Brain Following Cardiac Arrest: Outline of Experimental Design and Findings

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Type of experiment</th>
<th>Duration of cardiac arrest (min.)</th>
<th>Duration of maintenance</th>
<th>Remarks</th>
<th>Microangiographic observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (chest closed)</td>
<td>---</td>
<td>0 hr</td>
<td>Good filling of small arteries of the gray and white matter; moderate capillary filling in the gray matter</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Control (chest open)</td>
<td>---</td>
<td>4 hrs</td>
<td>Good filling of small arteries of the gray and white matter; moderate capillary filling in the gray matter</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Control (chest open)</td>
<td>---</td>
<td>5 hrs</td>
<td>Capillary filling seen in gray matter in spite of slightly defective arterial filling</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Control (chest open)</td>
<td>---</td>
<td>5 hrs</td>
<td>BP elevated briefly</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Control (chest open)</td>
<td>---</td>
<td>6 hrs</td>
<td>BP elevated 1 hour</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Short arrest &amp; long maintenance</td>
<td>3</td>
<td>5 hrs</td>
<td>BP temporarily elevated</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Long arrest &amp; short maintenance</td>
<td>12</td>
<td>10 min.</td>
<td>Only few large cerebral arteries filled (no-reflow)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Long arrest &amp; short maintenance</td>
<td>14</td>
<td>30 min.</td>
<td>Poor filling of arteries, no capillary filling</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Moderate arrest &amp; long maintenance</td>
<td>6</td>
<td>4 hrs</td>
<td>Good filling of small arteries of the gray and white matter; moderate capillary filling</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Moderate arrest &amp; long maintenance</td>
<td>9</td>
<td>4 hrs</td>
<td>Extensive arterial filling of both gray and white matter, poor capillary filling</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Long arrest &amp; long maintenance</td>
<td>12</td>
<td>4 hrs</td>
<td>Labile BP, cerebral angiography at 4 hours</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Long arrest &amp; long maintenance</td>
<td>12</td>
<td>5 hrs</td>
<td>Elevated BP for a 2-hour period, then lowered BP (spontaneous)</td>
<td></td>
</tr>
</tbody>
</table>

2-mm sections in coronal plane. The sections were sealed with paraffin wax to prevent drying, and radiographed in contact with Kodak High Resolution Plates using aFaxitron x-ray apparatus at 45 kV for microscopic observation of the brain vasculature.

Altogether, 12 dogs were used in the vital dye experiments. The experimental design in each individual case is presented in table 2. Fifteen milliliters per kilogram of fresh 1% Trypan blue solution in saline was infused intravenously ten minutes before stopping the circulation. The brain was removed and coronal sections were studied for gross extravasation of the dye. Fresh frozen 10 μ and 20 μ cryostat sections of various parts of the cortex were studied with a fluorescence microscope. Pieces of brain were also fixed in buffered 4% formaldehyde, embedded in paraffin, and stained with hematoxylin and eosin.

**Results**

**Microangiography**

Microangiographic observations on each control and experimental animal are briefly outlined in table 1.

In both completely untreated control and respirator-maintained dogs the introduction of a warm barium sulfate suspension into the ascending aorta resulted in filling of both the pial arteries and the intracerebral branches down to the precapillary level. Arteries of the white matter were well visualized as long, slender channels but very little or no capillary filling was seen in the white matter. Arteries of the gray matter, derived from the cortical branches like those of the white matter, were more numerous and more extensively filled than those of the white matter (fig. 1). The perforating cortical arteries of the gray matter have a smaller diameter and shorter course than those of the white matter. Some degree of capillary filling was also invariably seen in the cortical gray matter. With small magnification the capillary filling was seen as dots and small clouds of contrast (fig. 1A), but higher magnification was able to reveal filled individual capillaries (fig. 1B). No attempt was made to increase the degree of capillary filling from what occurred with infusion into the ascending aorta. It was also evident that the maintenance of the anesthetized, chest-open dog up to six hours did not influence microradiographic pattern of the cortical gray or white matter. The same was true in Dog No. 6, which was subjected to a short (three minutes) arrest and long maintenance.

Soon after long arrest the perfusability of the brain was poor; at ten minutes only a few large vessels were filled and capillary filling was lacking even at 30 minutes. Slow post-revival perfusion was also confirmed by carotid angiography in Dog No. 8. Angiography demonstrated a long arterial phase at ten minutes with improvement at 30 minutes after arrest.

In a dog (No. 9) which was subjected to a six-minute arrest and maintained for four hours, some capillary filling was observed, but in dogs (Nos. 11 and 12) arrested for 12
minutes and maintained for four and five hours, capillary filling of the gray matter was absent or negligible. Pial and perforating arteries as well as arteries of the white matter were filled in all these brains just as in the control brains (fig. 2).

Vital Dye Experiments

Results of the vital dye experiments are outlined in table 2. Control experiments with untreated and chest-open maintained animals established that no gross defect in microscopically observable capillary staining or no BBB damage was caused by the maintenance itself or by up to three consecutive cerebral angiographies (using 10 ml of Hypaque 60% contrast medium for each injection) (fig. 3). However, in a dog with marked prolonged hypertension (No. 3), slight pial and cortical parenchymal extravasation was observed. Infusion of the dye into the common carotid artery of dogs (Nos. 4 and 10) induced patchy staining throughout the brain, possibly as a result of increased vascular pressure. Poor capillary staining in fluorescence microscopy immediately after recovery, probably due to a no-reflow phenomenon, was demonstrated in Dog No. 7 by infusing Trypan blue immediately after arrest. Dogs Nos. 8 and 10, subjected to 7.5 minutes and 8.5 minutes of arrest, exhibited good red fluorescence of capillaries throughout the cerebral cortex after being maintained for four to five hours. Dogs Nos. 11 and 12, maintained for the same period but subjected to 11 to 14 minutes' arrest, did exhibit fewer stained capillaries in superficial cortical areas (fig. 4). This was considered to represent a decrease in the number of perfused capillaries in those areas. Evidence of BBB damage was seen in Dogs Nos. 9 and 12. Dog No. 9 had an unusually low blood pressure throughout the maintenance period of two hours and adequate reperfusion of the brain may not have occurred. The extent of dye extravasation was minimal, seen only with a microscope. Dog No. 12 was subjected to 14 minutes of arrest and was maintained for five hours. It presented small areas of pial dye extravasation, visible as a bluish hue to the naked eye, and a few intracerebral cortical perivascular fluorescent dye extravasations (fig. 5).

Discussion

The microangiographic technique practiced in the present experiments differs from passive postmortem perfusion previously used for the microangiographic study of brain cir-

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**Table 2** Trypan Blue Staining of Dog Brain Following Cardiac Arrest: Outline of Experimental Design and Findings

<table>
<thead>
<tr>
<th>Dog #</th>
<th>Type of experiment</th>
<th>Duration of cardiac arrest (min.)</th>
<th>Duration of maintenance</th>
<th>Remarks</th>
<th>Naked eye observations</th>
<th>Fluorescence microscopy findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control (chest closed)</td>
<td>--</td>
<td>--</td>
<td></td>
<td>No staining of brain tissue (normal)</td>
<td>Capillary cross sections, larger blood vessels, as well as meningeal structures fluorescent</td>
</tr>
<tr>
<td>2</td>
<td>Control (chest open)</td>
<td>5 hrs</td>
<td></td>
<td>3 carotid angiographies</td>
<td>Normal</td>
<td>Findings similar to No. 1</td>
</tr>
<tr>
<td>3</td>
<td>Control (chest open)</td>
<td>5 hrs</td>
<td></td>
<td>3 carotid angiographies</td>
<td>A few cortical bluish patches, no gross BBB damage, labile BP, then hypertension for 3 hrs</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Control (chest open)</td>
<td>4½ hrs</td>
<td>Trypan blue via common carotid artery</td>
<td>Patchy blue staining of brain</td>
<td>Dye extravasation, few capillary cross sections stained at outermost cortical area</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Control (chest open)</td>
<td>5½ hrs</td>
<td>Labile BP</td>
<td>Normal</td>
<td>Same as No. 1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Long arrest &amp; short maintenance</td>
<td>11½ hrs</td>
<td>Trypan blue given i.v. 2 min. before arrest</td>
<td>Normal</td>
<td>No dye extravasation, weak fluorescence</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Long arrest &amp; short maintenance</td>
<td>10½ hrs</td>
<td>Trypan blue injected i.v. 5 min. after recovery</td>
<td>One weak spot of bluish stain in cerebral cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Moderate arrest &amp; long maintenance</td>
<td>7½ hrs</td>
<td>Normal</td>
<td>No dye extravasation, normal capillary fluorescence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Moderate arrest &amp; long maintenance</td>
<td>8½ hrs</td>
<td>Low BP most of the time</td>
<td>Normal</td>
<td>Small areas of cortical extravasation of fluorescence</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Moderate arrest &amp; long maintenance</td>
<td>8 hrs</td>
<td>Trypan blue infused via common carotid artery; kidney failure</td>
<td>Random small patches of dye extravasation, less than in No. 4</td>
<td>Patchy areas of extravascular fluorescence</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Long arrest &amp; long maintenance</td>
<td>11 hrs</td>
<td>3 carotid angiographies</td>
<td>Normal</td>
<td>No extravasation of dye. Few perfused capillaries in outermost cortex</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Long arrest &amp; long maintenance</td>
<td>14 hrs</td>
<td>3 carotid angiographies</td>
<td>Patchy bluish hue cortically</td>
<td>Small areas of dye extravasation at brain's surface and within parenchyma. Few capillaries stained in the outermost cortex</td>
<td></td>
</tr>
</tbody>
</table>
Passive perfusion of the vascular bed may be used in order to obtain maximal filling of the capillary bed, but complete capillary visualization is not produced with any of the available variables of the technique. Recent studies using other organs suggest that by introducing Micropaque suspension with blood flow, as in the present experiment, information of the distribution of blood flow is obtained, and that this information may not be obtainable if passive perfusion is performed. With the present method the microangiographic filling of the terminal vascular bed occurs first, during the relatively undisturbed state of circulation. The ultimate degree of capillary filling in the brain is not necessarily related to the extent of gross arterial filling, the latter being determined mainly by the later course of infusion procedure. Our results in dog brain, which show better small vessel filling in the gray matter than in the white matter, are well correlated to physiological data which suggest higher blood flow in the gray matter.

Our angiographic and microangiographic data show impaired perfusion of the brain immediately following revival from cardiac arrest, also demonstrated by other investigators. While the circulation returned to normal range within 30 minutes to one hour, we found no evidence of marked postischemic hyperemia such as demonstrated by Hossmann et al. and Snyder et al. in animals immediately after recovery from cerebral ischemia.

**FIGURE 1.** Microangiogram on a control dog. (A) Coronal slice of the brain tissue radiographed on a High Resolution plate. Note the difference in the vascular filling of gray and white matter. Capillaries are seen as patchy densities in the gray matter. (X 3)
Delayed circulatory deterioration seems to be a phenomenon separate from the one reported to occur immediately following an anoxic attack.\textsuperscript{44} We interpret the lack of capillary filling in the late course of maintenance following cardiac arrest as suggestive of impaired capillary perfusion of the gray matter. Whether similar changes occur in the white matter cannot be concluded on the basis of microangiograms, due to the lack of capillary filling within the white matter with the perfusion method used. Trypan blue fluorescence suggests that reduction in the number of perfused capillaries after several hours' resuscitation mainly occurs in the most superficial layers of the cortex. Spector\textsuperscript{14} reported that a delay of about four hours was necessary to cause rise in cerebral water content and that a maximum increase was reached in 19 to 25 hours. The brain edema secondary to anoxic, ischemic or metabolic disturbances, called the cytotoxic type of brain edema, is considered to be predominantly due to increase of intracellular water content.\textsuperscript{28, 29} The mechanism of which has been a subject of several elaborate discussions.\textsuperscript{30, 39} Even if this type of edema may be generalized, local perfusion defects seen in fluorescence microscopy seem to occur which may be attributable to pressure effects since they are seen in the periphery of the brain tissue. Similar localizations of cortical perfusion defects were also visible in carbon black perfusion experiments.\textsuperscript{4}

Our results agree with numerous previous reports which indicate that transient cerebral ischemia will not induce extravasation of protein tracers through BBB. This is believed to be due to edematous swelling of the endothelial cells, which retain the dye even if permeability to glucose and ultrastructure show a severe damage to the cells.\textsuperscript{30, 41} In fact, there is evidence that a severe degree of ischemia may protect the BBB against chemically induced damage.\textsuperscript{32} The same reason may have caused less extensive extravasation of the dye in an ischemic brain (No. 10) than in a nonischemic (No. 4), both being damaged by intracarotid dye perfusion.

![Figure 2](http://stroke.ahajournals.org/Downloaded from http://stroke.ahajournals.org/)
It is noteworthy also that we did not find any damage to the BBB due to multiple angiographic examinations in post-ischemic state. It has been reported that in a limited damage of the BBB fluorescent dye may be seen in astrocytic processes even in the absence of visible extravasation of the dye. Such a phenomenon was not seen in our specimens, but the microscopic technique used may not have been adequate for detecting such subtle changes.

Analysis of our data shows that several complicating factors during resuscitation, like marked prolonged hypertension, extended hypotensive shock state or technical factors related to dye infusion, easily cause damage to BBB. Hypotension during the post-ischemic period is known to lead to irreversible brain damage in otherwise reversible conditions. Similarly, a prolonged hypertension, more than 90 mm Hg above the normal systolic pressure, will cause BBB damage. We only detected one case of slight dye extravasation in dogs resuscitated without detectable complicating factors and this was following 14 minutes of arrest.

We conclude that, in the experimental set-up used here, a no-reflow phenomenon does occur immediately after
resuscitation, and then cerebral blood flow returns to normal range within one hour. An impairment of capillary perfusion of the cortical layer without other morphological changes in the microangiographic pattern of the brain develops at three to four hours after a long cardiac arrest. Neither the arrest nor the secondary perfusion changes primarily involve BBB damage if measures for maintenance of circulation in physiological limits are carefully done. Less than ten minutes of arrest is not able to produce delayed circulatory disturbance observable with microangiographic or protein tracer methods within six hours of resuscitation.

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