Total Cerebral Ischemia: Application of a New Model System To Studies of Cerebral Microcirculation

DAVID L. JACKSON, M.D., PH.D., WILLIAM P. DOLE, M.D., JACK McGLOIN, B.S., M.S., AND JUHAD I. ROSENBLATT, PH.D.

SUMMARY Reduction in cerebral blood flow (CBF) following global ischemia has been implicated as a pathogenetic mechanism in progressive brain damage seen after restoration of effective cardiac action and cerebral perfusion pressure. There are serious limitations to many of the techniques for measuring regional cerebral blood flow, particularly during low flow states. In 15 dogs anesthetized with thiopental, 12 minutes of total cerebral ischemia (TCI) was produced using a double balloon occlusion technique. Total and regional cerebral blood flows were sequentially measured before and after balloon release by left ventricular injection of 15\(\mu\)m microspheres labelled with 5 different radionuclides. Total CBF was reduced 53 ± 5% (mean ± SEM) from pre-ischemic values between 1 and 3 hours after "resuscitation" despite normal perfusion pressure and arterial blood gases. CBF remained slightly reduced (24 ± 7%) at 6 hours post-ischemia. Thirty minutes after balloon release, grey matter flow was reduced 38 ± 8% from control values while adjacent white matter flow was increased 21 ± 10%. However, by 1 hour after ischemia, grey and white matter flows were both reduced (60 ± 3%, 41 ± 5% respectively). Similar differences in brain stem and cerebellar flow were also observed. The majority (71-86%) of the reduction in total CBF seen at one hour post-TCI is due to increased cerebrovascular resistance, with 14-29% of the decrease related to arteriovenous shunting.

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(15–20 mg/kg IV induction dose) over the first hour of the experiment. Additional anesthetic doses (1 mg/kg) were given to maintain light surgical anesthesia (no response to pain, cough reflex still present) until the induction of TCI. No additional anesthesia was required after the induction of cerebral ischemia. Dogs were intubated and respiration controlled on a volume respirator (Harvard apparatus Model 607). 

**Blood pressure** was maintained at 32 ± 1 mm Hg. Electrocardiogram, one-channel transparietal electroencephalogram, pulmonary artery capillary wedge pressure, central venous pressure, and left and right axillary artery blood pressures were recorded on a six-channel recorder (Brush Model 2600). All pressures were measured using standard pressure transducers (Statham, Model P23D). Cardiac output was determined in duplicate by the thermodilution technique (Instrumentation Laboratory Model 601). Arterial Pco2, PO2, and pH were measured on a blood gas analyzer (Instrumentation Laboratories, Inc. Model 301). In 4 animals early in the series, a 19 gauge plastic catheter (Instrumentation Laboratory Model 601). Arterial Pco 2, and pH were measured when the 4 microspheres were injected simultaneously. Microspheres were suspended in the 6% hydroxyethyl starch and 0.9% saline solution. 

Aggregation was minimized by adding 0.05% final concentration Tween-80. Before initial use, the stock suspension of microspheres was sonicated for 10 minutes and vortexed for 5 minutes before each use. Two to three ×10^7 spheres were withdrawn into a 5 cc plastic syringe with continual agitation maintained by flushing the sphere suspension back and forth between 2 syringes on a 3-way stopcock until injection. The spheres were injected over 60 seconds through a multi-holed left ventricular catheter inserted through the femoral artery. Its position was verified before and after injection by pressure tracing. An arterial reference sample was withdrawn through the left axillary artery using a Harvard pump and calibrated glass syringe at a withdrawal rate of 2.45 ± 0.01 cc/min. Withdrawal was begun 5 seconds before injection and continued for 90 seconds after completion of injection. The approach to microsphere use developed in this laboratory has been reported previously.

After the completion of each experiment, the animal was sacrificed by the intravenous injection of a saturated solution of KCL. The brain was rapidly removed and wet weight determined. The brain was fixed in buffered 10% sodium formaldehyde for 48 + hours. After fixation, the brain was divided into frontal, occipital, parietal, and anterior and posterior temporal lobes. Grey and white matter samples were separated from thin sections of each lobe. Left and right halves of the brain stem were separated into the diencephalon/basal ganglion, midbrain, pons, medulla, and cerebellum. The fixed tissue samples were minced and placed in preweighed counting vials and tissue sample weight determined. Calculation of blood flows based on wet tissue sample weight, fixed tissue sample weight, and dessicated dry weight all showed the same pattern of flow changes. "Wet sample" weights were used in calculating the flow data presented below. Arterial reference blood samples, pure isotope samples for fractional distribution of isotope activity,
and tissue samples were counted in a 3 channel gamma spectrometer (Beckman Model 300). Isotope separation and blood flow calculations were performed using a matrix algebra approach to the solution of simultaneous equations. Statistical analysis of grouped data was performed using analysis of variance on a statistical program utilizing a PDP 11-45 computer (Digital Equipment Company).

**Results**

Following aortic occlusion, the EEG became isoelectric within 20 to 30 seconds. Pupils were fixed and dilated and all reflexes abolished within one minute. After 12 minutes of circulatory arrest, administration of bicarbonate and hyperventilation restored mean arterial pressure to pre-ischemic level within 2 minutes. Occasional moderate (33%-50%) increases in mean arterial pressure were observed briefly following resuscitation, but returned to normal within 5 minutes. Intracranial pressure (ICP) was reduced during the ischemic period. There was an increase in ICP between 5 and 15 minutes following balloon release, but this returned to normal within 15-30 minutes and remained normal for the 6 hours following resuscitation. Arterial blood gases had returned to pre-ischemic values within 10-15 minutes after balloon release. Serial hematocrits, serum electrolytes, and osmolality were unchanged from pre-ischemic levels at each time for post-resuscitation flow determination (30-360 minutes post-TCI). Mean arterial blood pressure fell from a control mean of 135 to a mean of 120 over the 6 hour period, but the difference was not statistically significant. Cardiac output was reduced 40% at 1-3 hours post-resuscitation (p < 0.05). The ECG revealed sinus tachycardia without evidence of acute myocardial injury. Table 1 summarizes the hemodynamic/physiologic variables observed in the control period and over the 6 hours following 12 minutes of TCI. Thus, in this model system the major determinants of cerebral blood flow are at our near pre-ischemic levels during the measurement of post-ischemic cerebral blood flow. During microsphere injection and distribution, there were no significant changes in blood pressure, heart rate, ECG, or cardiac output. Control values for the pre-ischemic period for total and regional cerebral blood flow for the 15 experimental animals are shown in table 2. Total cerebral blood flow during the control period was 0.46 ml/gm/min. Grey matter flow was 0.55 ml/gm/min while white matter flow was 0.37 ml/gm/min. This was remarkably constant in all lobes of the cerebral cortex for both grey and white matter. Serial changes in total cerebral blood flow following balloon release after 12 minutes of circulatory arrest are shown in figure 1. Total cerebral blood flow was slightly decreased ½ hour after balloon release (0.28 ml/gm/min) though not statistically significantly different from control values. However, 1 hour following balloon release, total cerebral flow has decreased to 0.22 ml/gm/min (53 ± 4%) mean % original value ± SE). This was significantly lower than control flow (p < 0.005). This was observed despite maintenance of cerebral perfusion pressure and control pre-ischemic blood gases values. Cerebral blood

<p>| Table 1. Hemodynamic and Physiologic Parameters After Resuscitation From 12 Minutes &quot;Circulatory Arrest&quot; |</p>
<table>
<thead>
<tr>
<th>No of Dogs (10)</th>
<th>Pre-Ischemic (mean ± SEM)</th>
<th>Post-Ischemic (min) (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>128 ± 6</td>
<td>142 ± 8</td>
</tr>
<tr>
<td>Mean Arterial (mm Hg)</td>
<td>132 ± 8</td>
<td>126 ± 8</td>
</tr>
<tr>
<td>Pulmonary Capillary Wedge Pressure (mm Hg)</td>
<td>7 ± 3</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>Cardiac Output (L/min)</td>
<td>1.9 ± .3</td>
<td>1.7 ± .25</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± .03</td>
<td>7.33 ± .02</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>32 ± 1</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>Po2</td>
<td>88 ± 4*</td>
<td>96 ± 6**</td>
</tr>
</tbody>
</table>

*Room Air  **4 l. Supplemental Oxygen Flow

<p>| Table 2. Pre-Ischemic Total and Regional Cerebral Blood Flow |</p>
<table>
<thead>
<tr>
<th>Blood Flow cc/min/gm</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Brain</td>
<td>0.46 ± .04</td>
</tr>
<tr>
<td>Cerebrum</td>
<td>0.55 ± .03</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>0.34 ± .03</td>
</tr>
<tr>
<td>White Matter</td>
<td>0.34 ± .03</td>
</tr>
<tr>
<td>Brain Stem</td>
<td>0.35 ± .02</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.45 ± .04</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.48 ± .03</td>
</tr>
<tr>
<td>Pons</td>
<td>0.48 ± .03</td>
</tr>
<tr>
<td>Diencephalon</td>
<td>0.47 ± .02</td>
</tr>
<tr>
<td>Midbrain</td>
<td>0.47 ± .02</td>
</tr>
</tbody>
</table>
Changes in total cerebral blood flow following 12 minutes total cerebral ischemia (TCI).

Changes in regional grey matter flow following 12 minutes TCI.

Changes in regional white matter flow following 12 minutes TCI.

38 ± 8% (p < 0.05), while adjacent white matter flow was still increased (21 ± 10%, p = 0.1). One hour after TCI, both grey and white matter flows were reduced (60 ± 3%; p < 0.005 and 41 ± 5%; p < 0.01 respectively). Grey and white matter flow remained diminished 3 hours after TCI (57 ± 5% and 47 ± 5% respectively, p < 0.005). Blood flow increased toward control values at 6 hours after TCI (67 ± 8 and 69 ± 7% of the control values). In the cerebellum, blood flow increased from 0.35 ml/gm/min during the control period to 60 ml/gm/min ½ hour after ischemia (p = 0.05) (fig. 4). Regional flow in the midbrain, pons, and medulla demonstrated a pattern similar to that seen in grey matter with a 40% decrease in flow at ½ hour and a 61% decrease 1 hour after TCI (fig. 5).

Measurement of a decrease in regional CBF after TCI with 15/μ microspheres could, in part, be related to "shunting" of these microspheres from the arterial to the venous circulation. To evaluate the possible contribution of shunting of 15/μ microspheres to the decrease in flows measured after TCI, 15 and 50 micron spheres labelled with different radioisotopes...
were simultaneously injected in 6 dogs. These 2 injections were made in 2 control, non-ischemic animals, in 2 animals ½ hour following TCI, and in 2 animals 1 hour following TCI. Total cerebral blood flow was then calculated for each animal independently for each sphere size. A ratio of the flow rate calculated from the 15μm microsphere data to the flow calculated from 50μm microsphere data was then calculated. In the control animals, the ratio of 15μ/50μ flow was 0.95 and 0.96. At ½ hour following global ischemia, the ratio was 0.79 and 0.92. One hour following global ischemia, the 15/50 ratio was 0.71 and 0.86. Thus, at the point of maximally decreased blood flow calculated by 15μ microspheres (1 hour post-ischemia), approximately 14 to 29% of the decrease in the blood flow calculated from 15μ microspheres can be attributed to the shunting of the 15μ microspheres through newly opened "arteriovenous thoroughfares" between 15 and 50μ in diameter. In the dog, "pure" cerebral venous blood cannot be obtained without intracranial surgery (see Discussion). However, no 50μ spheres were detected in samples of jugular venous blood withdrawn during and for 3 minutes after the simultaneous microsphere injections.

**Discussion**

Despite considerable effort, the basic mechanism underlying progressive central nervous system damage following resuscitation from total circulatory arrest remains unclear. Ames and his co-workers suggested a pathological basis for the "no-reflow" or impaired reperfusion phenomenon based on endothelial blebs and edema of the glial foot processes causing intrinsic compression of the capillaries within the central nervous system. Snyder and his colleagues subjected anesthetized dogs following thoracotomy to 15 minutes of global ischemia induced by aortic cross-clamping and demonstrated a 50% reduction in cortical blood flow 40–120 minutes after resuscitation using xenon clearance techniques. Some of these dogs required vasoressor (norepinephrine) infusion to maintain arterial pressure at pre-ischemic levels. In this study, post-ischemic hypoperfusion, which developed by 30–60 minutes after TCI, was not associated with any increase in intracranial pressure. Hosmann and his colleagues reported a 50 to 75% reduction in post-ischemic cerebral blood flow, again utilizing xenon clearance techniques, in anesthetized rats subjected to 30–60 minutes of circulatory arrest produced by clamping of the aortic arch combined with trimethaphan-induced hypotension. Vasoactive drugs were required to maintain post-ischemic perfusion pressure. The extensive intrathoracic surgery in both these studies and the use of vasoactive agents complicate the interpretation of the post-ischemic blood flow data. Lin and his colleagues reported decreased cerebral blood flow using radiolabelled microspheres technique in a model combining open thoracotomy, ventricular fibrillation, aortic cross-clamping, and open chest cardiac massage during the period of cross-clamping. The effect of light barbiturate anesthesia in many studies, including our own, is an important factor to consider in assessment of cerebral blood flow data. However, since cerebral blood flow is diminished by barbiturate anesthesia in non-ischemic animals, use of thiopental anesthesia prior to the induction of global ischemia would decrease control flows, particularly in the grey matter. This would act to decrease any difference noted between control values and post-ischemic values. In our model, no anesthesia is administered after the induction of cerebral ischemia.

The use of xenon (133Xe) and other gas clearance methodologies for the measurement of cerebral blood flow has been challenged for low flow states. Diffusion bypass of 133Xe between arteries and veins has been demonstrated in anesthetized dogs. This could contribute to a small underestimation of cerebral blood flow, particularly in low flow states. Watz has shown that the washout curves in many experimental animal species can not always be readily resolved into a bimodal pattern, with a fast component representing grey matter flow rates and a slow component representing white matter flow rates. "Total" cerebral blood flow can be calculated using a non-compartmental approach. It is, however, unclear what, if any, relationship these flow data bear to grey or white matter regional CBF.

In our present studies, 15μ microspheres radio-labelled with 5 different isotopes were used to measure regional cerebral blood flow during control periods and after 12 minutes of total cerebral ischemia in lightly thiopental anesthetized dogs. Major determinants of cerebral blood flow (cerebral perfusion pressure Pco2, pH, Po2) were not significantly different in the post-ischemic period from the pre-ischemic control values. The validity of 15μ microspheres as a technique to measure cerebral regional blood flow has been previously established. The 53% reduction in total cerebral blood flow 1 hour following 12 minutes of total cerebral ischemia in this study is similar to the 50% decrease seen post-ischemia in cortical blood flow in the dog reported by Snyder. This reduction in cerebral blood flow
without any significant change in perfusion pressure usually indicates increased cerebrovascular resistance. However, post-ischemic flows measured with 15μm microspheres may be underestimated if that size sphere passes through low resistance vascular channels (“arteriovenous thoroughfare”) instead of being trapped at the capillary and small arterial level, as in the pre-ischemic state.22 The cerebral circulation in the dog does have arteriovenous anastomotic channels, more numerous, but appearing similar on anatomic studies to arteriovenous connections seen in primates.23 The standard methodology for measuring arteriovenous shunting with microspheres requires a sample of pure venous effluent from the organ under study. Since numerous vascular communications between extracranial and intracranial circulation occur in the dog, obtaining a pure sample of cerebral venous blood is technically difficult without invasive neurosurgical intervention.24 In this study, the effect of sphere loss through the opening of dilated vascular channels or true arteriovenous anastomosis (>15μ but <50μ) was studied non-invasively by comparing blood flow calculated from both 15μm microspheres and 50μm microspheres injected simultaneously. In the control state, the blood flow calculated from the 50μm sphere data is 4 to 5.5% higher than the value from the 15μm sphere data. This is likely accounted for by rheologic differences between the 50 and 15μm spheres, as well as the possibility of some low level of shunting during the control period. No spheres were detected in the jugular venous blood samples obtained during the control injections. Also, no 50μm spheres were detected in jugular venous blood in the post-ischemic period, although jugular venous blood in the dog is a mixture of (predominantly) extracranial and intracranial venous blood. The absence of any 50μm spheres is at least suggestive that any arteriovenous shunts that were present were in either extra- or intracranial circulation were not larger than 50μ in diameter. From the data on simultaneous injection of 15 and 50μm microspheres, it appears that approximately 15 to 30% of the reduction of cerebral blood flow measured by 15μm microspheres at 1 hour after TCI can be accounted for by opening of “arteriovenous thoroughfares” or arteriovenous anastomosis in the 15–50μ diameter size range. However, the major factor 70–85%) in the reduction in cerebral blood flow measured post-ischemia with 15μm microspheres cannot be accounted for by shunting using this technique. This demonstration of the major role played by increased cerebral vascular resistance in the delayed (30–60 min post-TCI) development of the post-ischemic impaired reperfusion supports the use of microsphere CBF data in this study. We believe that this portion of the post-ischemic reduction in flow measured with 15μm microspheres represents an increase in cerebrovascular resistance at the resistance vessel level.

The data on regional CBF and A-V shunting following global cerebral ischemia strongly suggest that the alterations in cerebral blood flow are primarily mediated by heterogeneous changes in small vessel cerbrovascular resistance. The mechanism of such resistance changes remains unsettled. A number of mechanisms have been proposed, including glial edema and capillary endothelial “blebs”,25 disseminated intravascular coagulation,26 and localized vasoconstriction secondary to extracellular fluid hyperkalemia (Wade et al., 1975). The increase in blood flow in the white matter and cerebellum ½ hour post-ischemia while brain stem structures and gray matter flows are reduced suggests a difference in the time course in these tissues for resolution of the initial post-resuscitation hyperemia noted in a number of cerebral ischemia model systems.19,20 This may be related to structural differences between gray and white matter, including the cellular and capillary density. It is clear from more recent work in Ames’ laboratory27 that extrinsic compression of the capillaries by either endothelial blebs or glial foot process swelling does not occur in a time course or to an extent consistent with the regional cerebral blood flow changes seen in this study. Direct visualization of pial arterioles in the post-ischemic period has shown early vasodilation followed by vasoconstriction.27

The occurrence of impaired cerebral perfusion following restoration of perfusion pressure after total cerebral ischemia has led to the suggestion that there is a potentially reversible component to the central nervous system damage seen following cardiac arrest. If the final neurologic outcome is not based totally on the immediate neuronal ischemic damage occurring during the period of circulatory arrest, the continuing ischemia related to the observed increase in cerebrovascular resistance may be amenable to therapeutic intervention. There are experimental data in animal models that post-ischemic reduction in cerebral blood flow is associated with poor neurologic outcome.28 Therapy designed to improve cerebral microcirculation has been reported to ameliorate neurologic damage following 12 minutes of ventricular defibrillation in dogs.7 The data presented in this study suggest that therapy designed to reduce cerebrovascular resistance in the small arterial resistance vessels might increase nutritive cerebral blood flow in the post-ischemic period. Whether increased cerebral blood flow during the impaired reperfusion period will improve neurologic outcome or be associated with an exacerbation of the cerebral edema which develops later in the national history of severe global cerebral ischemia remains to be examined.

References
Total cerebral ischemia: application of a new model system to studies of cerebral microcirculation.

D L Jackson, W P Dole, J McGloin and J I Rosenblatt

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