Unilateral Antegrade Cerebral Perfusion Through the Right Axillary Artery Provides Uniform Flow Distribution to Both Hemispheres of the Brain

A Magnetic Resonance and Histopathological Study in Pigs

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Background—Bilateral antegrade cerebral perfusion (ACP) has decreased in popularity over the past decade because of its complexity and the risk of cerebral embolism. We used magnetic resonance (MR) perfusion imaging to assess flow distribution in both hemispheres of the brain during unilateral ACP through the right carotid artery via a cannula placed in the right axillary artery in conjunction with hypothermic circulatory arrest.

Methods and Results—Twelve pigs were randomly exposed to 120 minutes of either bilateral ACP through both carotid arteries (n=6) or unilateral ACP through the right axillary artery (n=6) at pressures of 60 to 65 mm Hg at 15°C, followed by 60 minutes of cardiopulmonary bypass at 37°C. MR perfusion images were acquired every 30 minutes before, during, and after ACP. The brain was perfusion fixed for histopathology. During initial normothermic cardiopulmonary bypass, MR perfusion imaging showed a uniform distribution of flow in the brain. In both the bilateral and unilateral ACP groups, the same pattern was maintained, with an increase in regional cerebral blood volume during ACP and reperfusion. The changes in regional cerebral blood volume and mean transit time were similar in both hemispheres during and after unilateral ACP. No difference was observed between the 2 groups. Histopathology showed normal morphology in all regions of the brain in both groups.

Conclusions—Both bilateral ACP and unilateral ACP provide uniform blood distribution to both hemispheres of the brain and preserve normal morphology of the neurons after prolonged hypothermic circulatory arrest. (Circulation. 1999;100[suppl II]:II-309–II-315.)

Key Words: magnetic resonance imaging ▪ brain ▪ cerebrovascular circulation ▪ perfusion
monitor the distribution of blood, regional cerebral blood volume (rCBV), and mean transit time (MTT) before, during, and after HCA with either bilateral or unilateral ACP. MR perfusion imaging has been shown to be suitable for monitoring blood distribution in the brain noninvasively in our pig model. Histopathology was performed at the end of the experiments.

Methods

Twelve young female pigs (weight, 32 to 38 kg; age, ~105 days) that seemed to be neurologically mature13 were acclimatized in the animal facility of the Institute for Biodiagnostics for ≥12 days before the study and fasted for 12 hours before operation. All pigs received humane care in compliance with the guidelines of the Canadian Council on Animal Care.

Surgical Preparation

As described previously,6,7,9 preanesthesia was induced with midazolam (0.3 mg/kg IM), ketamine (20 mg/kg IM), and atropine (0.02 mg/kg). After endotracheal intubation, the pig was ventilated mechanically with 60% oxygen and 40% air. The ventilator rate and tidal volume were adjusted to maintain the arterial CO2 tension between 35 and 45 mm Hg. Anesthesia was maintained with 1.5% to 2.0% isoflurane. A temperature probe was placed in the esophagus to monitor core temperature. Urine output was collected through a bladder catheter.

The chest was opened via a median sternotomy. The right axillary artery was isolated, and a 12F modified double-lumen retrograde cannula was placed in the right axillary artery. The left axillary artery, common carotid arteries, and internal mammary artery were occluded. A small cannula was placed in the axillary artery near the aortic arch; unilateral ACP was established by opening the axillary artery with occlusion of the brachiocephalic artery and the left carotid artery. Perfusion pressure was monitored continuously and maintained at 60 to 65 mm Hg in the common carotid artery during both unilateral and bilateral ACP. No inotropic support was used during the experiments.

MR Perfusion Imaging

All experiments were performed on a Bruker 7-T, 40-cm-bore MR instrument equipped with actively shielded gradient coils with a specially designed 12-cm-diameter dual-ring surface radiofrequency coil (transmit/receive). A single-slice, gradient-echo, fast, low-angle shot technique (field of view, 12 cm; slice thickness, 5 mm; echo time, 10 ms; repetition time, 15 ms; 128×64 matrix; total acquisition time, 952 ms per image) was used to acquire T2*-weighted images. Expiratory breathing was maintained throughout the experiments, and shimming the magnetic field were completed, normothermic CPB was initiated in the pigs.

The CPB circuit consisted of Cobe roller pumps (model C22.2), Dideco D733), water bath (Lauda MGW type RMSG), and a membrane oxygenator (Cobe Optima) with integrated heat exchanger. The circuit was primed with 700 mL lactated Ringer’s solution, 500 mL Pentaspan, 25 mL of 1 mol/L sodium bicarbonate, and 5000 IU heparin. When necessary, sodium bicarbonate was given to maintain arterial blood pH within the normal range of 7.35 to 7.45. The alpha-stat approach was used during hyperthermia. Arterial blood gases were monitored and measured at 37°C with a blood gas analyzer (Stat 9, NOVA Biomedical, USA). Blood electrolytes, glucose, and osmolality were monitored and kept within normal ranges.

Experimental Groups and Protocol

The pigs were randomly assigned to 1 of the following groups: group 1, deep HCA plus bilateral ACP (B-ACP; n = 6); group 2, deep HCA plus unilateral ACP (U-ACP; n = 6). The experimental protocol is shown in Table 1.

After preparations for the MR studies (ie, positioning the head and shimming the magnetic field) were completed, normothermic CPB was initiated in the pigs. During CPB, pump flow was maintained at 100 mL · kg⁻¹ · min⁻¹ (Cobe HVFR 3700), arterial filter (40 micro, iodoce D733), water bath (Lauda MGW type RMSG), and a membrane oxygenator (Cobe Optima) with integrated heat exchanger. The circuit was primed with 700 mL lactated Ringer’s solution, 500 mL Pentaspan, 25 mL of 1 mol/L sodium bicarbonate, and 5000 IU heparin. When necessary, sodium bicarbonate was given to maintain arterial blood pH within the normal range of 7.35 to 7.45. The alpha-stat approach was used during hyperthermia.

Arterial blood gases were monitored and measured at 37°C with a blood gas analyzer (Stat 9, NOVA Biomedical, USA). Blood electrolytes, glucose, and osmolality were monitored and kept within normal ranges.

<table>
<thead>
<tr>
<th>TABLE 1. Experimental Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Action</td>
</tr>
<tr>
<td>Cooling</td>
</tr>
<tr>
<td>Temperature, °C</td>
</tr>
<tr>
<td>Duration, min</td>
</tr>
<tr>
<td>R-CPB indicates reperfusion with cardiopulmonary bypass.</td>
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</tbody>
</table>

Circulatory arrest was achieved when the body temperature reached 15°C. At the same time, bilateral ACP was performed by opening the line in the axillary artery with the occlusion of the brachiocephalic artery near the aortic arch; unilateral ACP was established by opening the axillary artery line with occlusion of the brachiocephalic artery and the left carotid artery. Perfusion pressure was monitored continuously and maintained at 60 to 65 mm Hg in the common carotid artery during both unilateral and bilateral ACP. No inotropic support was used during the experiments.

Tissue Preparation

At the end of each experiment, the brain was perfused under anesthesia with cold heparinized saline through the carotid arteries to wash blood from the brain. This was followed by perfusion and immersion fixation with 10% buffered formaldehyde solution. The same cross sections used for MR imaging were identified from anatomical markers and selected for paraffin embedding. Other anatomical areas of interest, including cingulate and temporal cortex, striatum, the hippocampus, thalamus, cerebellum, caudate nucleus, and proximal spinal cord, were also obtained for paraffin embedding. All samples were cut into 5-μm-thick slices. Hematoxylin and eosin staining was performed. Injury severity was based on the number of damaged neurons in each area. The minimum criteria for diagnosis of ischemic neuronal damage included mild cytoplasmic eosinophilia, shrunken neurons with scalloping of the margins, and nuclear changes consisting of coarse nuclear chromatin or pyknosis. Injury was graded (0 to 5) on the basis of the number of damaged neurons within each selected region: 0 = normal; 1 = <10%; 2 = 10% to 25%; 3 = 26% to 50%; 4 = 51% to 75%; and 5 = >75%. Neurons were...
counted by use of a rectangular ocular graticule at an ocular magnification of 400×. It has been demonstrated that neuronal damage can be detected 60 minutes after ischemic/hypoxic insults in our acute pig model.3

Statistical Analysis
All data are presented as mean±SEM. A repeated-measures ANOVA and Duncan’s multiple range test were used for comparison between different time points, 2 hemispheres, and different regions of the brain within a group, as well as between 2 groups, and P<0.05 was considered significant.

Results

General Conditions
The pigs were randomly assigned to either group before each experiment. No inotropic drugs were used during any of the experiments to avoid any effect of the drugs on cerebral blood flow. The time for cooling and rewarming was 30 to 35 minutes and 35 to 40 minutes, respectively. There were no significant differences in temperature, arterial blood gases, hematocrit, mean arterial pressure, and pump flow between the 2 groups (Table 2). No hemorrhage was observed in the brain of any pig from either group.

Distribution of Blood in the Brain
Uniform flow distribution to the entire brain was observed in all pigs from both groups during initial normothermic CPB. rCBV increased in most regions of the brain during both bilateral and unilateral ACP and remained high 60 minutes after normothermic reperfusion with CPB. Only a few small areas showed reduced rCBV (Figure 1). In the U-ACP group, there was no significant difference in rCBV between the left and right hemispheres of the brain in the 5 selected regions during either unilateral ACP or at 60 minutes of reperfusion with normal CPB (Figure 2). These data indicate that both hemispheres received the same amount of blood during unilateral ACP. The pattern of change in rCBV was similar in the B-ACP and U-ACP groups. No significant difference in rCBV was observed between the B-ACP and U-ACP groups in any region of either hemisphere during ACP (Figure 3) or 60 minutes after reperfusion with normal CPB. Sixty minutes after reperfusion, the total area showing poor perfusion was very small in both the B-ACP and U-ACP groups (Figure 1 and Table 3), which is consistent with our previous findings.9 There was no significant difference in the total amount of poorly reperfused tissue in the right and left hemispheres of the brain in the U-ACP group. There was also no significant difference in these parameters between the 2 groups (Table 3).

MTT in the Brain
During normothermic CPB, MTT was 7 to 10 seconds throughout the brain (Figure 4). This indicates that the time required for blood to pass through the tissue was uniform throughout the brain. MTT was significantly prolonged in all brain sections during ACP (unilateral and bilateral) at 15°C (2 to 3 times longer than the baseline level obtained during initial normothermic CPB at 37°C), indicating a significant decrease in the speed of blood passing through the brain tissue. A similar prolongation of MTT was observed during CPB at 15°C. MTT recovered to its baseline level in both groups during normothermic reperfusion with CPB. In the U-ACP group, there was no significant difference in MTT between the left and right hemispheres in any brain region during either ACP or reperfusion with CPB (Figure 5). In both the B-ACP and U-ACP groups, MTT was similar in all regions of the brain during ACP and reperfusion with CPB (Figures 3 and 4).

Histopathology
Normal morphology of the neurons was observed in all regions of the brain in both the B-ACP and U-ACP groups. More specifically, there was no observable neuronal damage in the left hemisphere in the U-ACP group. The grade of neuronal damage across the brain ranged from 0 to 0.3 in both groups. No hemorrhage was observed in the brain of any pig from either group.

Discussion
To the best of our knowledge, this is the first report on flow distribution observed in real time during unilateral ACP

### TABLE 2. Temperature, Blood Gases, Hematocrit, Mean Arterial Pressure, and Pump Flow in Pigs

<table>
<thead>
<tr>
<th>Group</th>
<th>37°C CPB (Baseline)</th>
<th>15°C CPB</th>
<th>End of 15°C ACP</th>
<th>15°C R-CPB</th>
<th>37°C R-CPB</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-ACP</td>
<td>37.1±0.2</td>
<td>15.0±0.1</td>
<td>14.9±0.1</td>
<td>14.8±0.2</td>
<td></td>
</tr>
<tr>
<td>U-ACP</td>
<td>37.1±0.2</td>
<td>14.8±0.1</td>
<td>14.7±0.1</td>
<td>15.1±0.3</td>
<td>37.4±0.1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.44±0.04</td>
<td>7.46±0.01</td>
<td>7.37±0.02</td>
<td>7.42±0.03</td>
<td>7.38±0.02</td>
</tr>
<tr>
<td>B-ACP</td>
<td>7.44±0.02</td>
<td>7.44±0.03</td>
<td>7.43±0.04</td>
<td>7.44±0.02</td>
<td>7.38±0.03</td>
</tr>
<tr>
<td>U-ACP</td>
<td>39.5±1.4</td>
<td>34.1±2.2</td>
<td>45.7±2.0</td>
<td>41.7±2.4</td>
<td>42.8±3.4</td>
</tr>
<tr>
<td>B-ACP</td>
<td>39.6±2.3</td>
<td>37.4±3.0</td>
<td>43.3±4.9</td>
<td>37.3±2.1</td>
<td>41.0±1.7</td>
</tr>
<tr>
<td>U-ACP</td>
<td>16.7±0.6</td>
<td>12.7±0.4</td>
<td>11.5±0.4</td>
<td>12.0±0.7</td>
<td>15.7±0.4</td>
</tr>
<tr>
<td>B-ACP</td>
<td>15.8±0.7</td>
<td>12.8±0.9</td>
<td>12.8±0.8</td>
<td>12.5±0.4</td>
<td>14.0±1.1</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>61.8±4.2</td>
<td>67.7±4.3</td>
<td>64.5±1.7</td>
<td>44.3±5.6</td>
<td>62.0±4.2</td>
</tr>
<tr>
<td>B-ACP</td>
<td>62.0±5.3</td>
<td>61.2±8.1</td>
<td>62.5±2.2</td>
<td>47.3±6.5</td>
<td>57.2±3.8</td>
</tr>
<tr>
<td>Pump flow*</td>
<td>100</td>
<td>100</td>
<td>423±42</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>B-ACP</td>
<td>100</td>
<td>100</td>
<td>508±88</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>U-ACP</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

R-CPB indicates reperfusion with CPB. Values are mean±SEM. No significant difference was observed between groups in any parameter at any stage of the experiments.

*Unit of measure is mL · kg⁻¹ · min⁻¹ during CPB and mL/min during ACP.
through the right axillary artery. The present study clearly demonstrates that unilateral ACP through the right axillary artery provides equal and uniform flow distribution to both hemispheres of the brain. During unilateral ACP, the speed of blood passing through the brain tissue is also similar in both hemispheres of the brain. The pattern of change in rCBV and MTT was similar in the U-ACP and B-ACP groups. These findings indicate that during unilateral ACP the left hemisphere receives an amount of blood similar to that of the right hemisphere through collateral circulation, particularly through the circle of Willis. As a consequence of the uniform flow distribution to the entire brain during unilateral ACP, no morphological damage to the brain was observed. The flow supplied by unilateral ACP appears to be sufficient to maintain normal energy metabolism in both hemispheres of the brain, as determined by localized 31P-MR spectroscopic imaging (Dr Ye et al, unpublished data, 1998).

Neurological injury remains a serious consequence after cardiac surgery requiring circulatory arrest (particularly for times >45 minutes) despite the protection offered by

Figure 1. Representative rCBV maps showing difference between initial CPB at 37°C and ACP at 15°C and 60 minutes of reperfusion with CPB (R-CPB) at 37°C. Top, B-ACP; bottom, U-ACP. Red indicates that rCBV is lower than during initial CPB; black, rCBV is same as during initial CPB; and other colors, rCBV is greater than during initial CPB.

Figure 2. Percentage changes in rCBV in right and left hemispheres during U-ACP at 15°C and at 60 minutes of reperfusion with CPB (R-CPB) at 37°C. No significant difference was observed between right and left hemispheres.

Figure 3. Left, Percentage change in rCBV in different brain regions during ACP in B-ACP and U-ACP groups. No significant difference was observed between groups. Right, MTT in different brain regions during ACP in B-ACP and U-ACP groups. No significant difference was observed between groups.
hypothermia. Our previous studies\textsuperscript{6–8} have demonstrated that conventional bilateral ACP completely preserves normal metabolites (ATP and phosphocreatine), intracellular pH, morphology, and structural protein (microtubule-associated protein 2) after 2 hours of HCA. Retrograde cerebral perfusion does not maintain normal metabolite levels or intracellular pH during prolonged HCA and results in ischemic damage to brain tissue and loss of structural protein. ACP may be the best choice for brain protection, particularly when it is difficult to estimate preoperatively the time required for surgical procedures.

The cephalic vessels used for ACP differ among hospitals; however, both the left common carotid and right common carotid arteries are usually used. Frist et al\textsuperscript{18} and Wozniak et al\textsuperscript{19} reported that unilateral cerebral perfusion through the brachiocephalic or innominate artery was sufficient to prevent cerebral ischemia in humans. However, direct cannulation of the cephalic arteries is still required in their techniques. Unilateral ACP through a catheter placed in the axillary artery avoids direct cannulation of the cephalic vessels. Direct cannulation may damage the cephalic vessels and increase the risk of cerebral embolism resulting from detachment of atherosclerotic debris.\textsuperscript{20,21} Unilateral ACP through the right axillary artery may be particularly suitable in patients with severe iliofemoral atherosclerotic disease, because the cannula in the right axillary artery can be used for both CPB and ACP. Possible morbidity directly related to cannulation of the axillary artery may include axillary artery thrombosis and brachial plexus injury. However, according to a report from the Cleveland Clinic Foundation,\textsuperscript{12} these complications appear to be low. Of the 35 patients in that study using the axillary

\begin{table}
\centering
\caption{Total Area of Reduced rCBV (Percent of Entire Image Area)}
\begin{tabular}{|c|c|c|c|c|c|c|c|}
\hline
\textbf{Brain Hemisphere} & \textbf{During ACP} & \multicolumn{4}{|c|}{\textbf{60 min of Reperfusion With CPB}} \\
& & 0–25 & 26–50 & 51–75 & 76–100 & 0–25 & 26–50 & 51–75 & 76–100 \\
\hline
\textbf{B-ACP} & & & & & & & & & \\
Right & 1.2±0.5 & 3.2±2.1 & 6.0±3.9 & 1.2±1.2 & 5.8±2.3 & 2.5±1.2 & 0.2±0.2 & 0 \\
Left & 2.2±1.8 & 1.3±0.8 & 2.5±2.5 & 1.8±1.8 & 2.3±0.8 & 0.8±0.4 & 0 & 0 \\
\textbf{U-ACP} & & & & & & & & & \\
Right & 1.7±1.1 & 4.2±2.7 & 3.2±2.2 & 0.7±0.7 & 3.2±1.3 & 1.2±0.6 & 0.5±0.3 & 0 \\
Left & 3.3±2.0 & 4.2±2.7 & 3.0±2.0 & 1.0±1.0 & 2.0±1.3 & 0.8±0.7 & 0.2±0.2 & 0 \\
\hline
\end{tabular}
\end{table}

Values are mean±SEM. No significant difference was observed between the right and left hemispheres in either the B-ACP or U-ACP group. No significant difference was observed between the B-ACP and U-ACP groups.

\*Levels of reduction relative to the baseline level obtained during initial normothermic CPB.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mean_transit_time.png}
\caption{Mean Transit Time}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mtt_maps.png}
\caption{Representative MTT maps obtained during initial CPB (37°C), ACP at 15°C, and at 60 minutes of reperfusion with normothermic CPB (37°C R-CPB). Top, B-ACP; bottom, U-ACP.}
\end{figure}
artery for CPB, 1 patient who had 3 previous cardiac operations and underwent axillary artery cannulation had axillary artery thrombosis, and another patient had mild residual numbness in the hand. No patient showed any cerebrovascular injury or evidence of arterial embolic events.

Our results were obtained with normal pigs. These results cannot be completely translated to all clinical situations, particularly to patients who have severe atherosclerotic disease of the cerebral arteries. In the study of Wozniak et al. of 25 consecutive patients undergoing surgical treatment of an aneurysm, no patient was considered unsuitable for unilateral ACP through the innominate artery as a result of test perfusion. All 25 patients survived the operative treatment. Although complete blockage or lack of collaterals between both hemispheres may be uncommon, special preoperative evaluation may be necessary to exclude the presence of important carotid disease and to confirm the patency of the circle of Willis. MR angiography, aortography, digital subtraction angiography, or CT could be used for this evaluation.

The measurement of real-time blood distribution in the brain remains challenging because most methods, such as microspheres, hydrogen clearance, and laser-Doppler flowmetry, are limited to local flow measurements or in the number of measurements. Dynamic contrast-enhanced MR perfusion imaging can provide repetitive, noninvasive measurements of relative rCBV and tissue perfusion, as well as MTT in real time. The hyperperfusion observed in the present study during ACP and early after ACP is consistent with our previous results. The significant prolongation in MTT observed during bilateral and unilateral ACP at 15°C appears to be due to hypothermia because the same prolongation of MTT was also observed during CPB at 15°C. This prolongation in MTT may be due to increased blood viscosity, dilatation of vessels, a greater opening of the microvasculature, and increased blood volume during deep hypothermia.

Because of possible differences in the anatomy of the vascular system between humans and animals, the data obtained with this model cannot be completely translated into the clinical situation. However, animal models provide controlled experimental conditions and allow measurements that often are not feasible in humans. The present study provides the first detailed report on regional blood distribution in both hemispheres of the brain during HCA and prevent ischemic injury in both hemispheres in the normal pig. The right axillary technique may be the method of choice for brain protection during elective surgery requiring a relatively long period of HCA.

Acknowledgments

This work was supported by the National Research Council of Canada and the Heart and Stroke Foundation of Manitoba (grant). We would like to thank Monique St-Jean, Lori Gregorash, Rachelle Mariash, and Shelly Germisch for technical assistance.

References


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Circulation. 1999;100:II-309-II-315
doi: 10.1161/01.CIR.100.suppl_2.II-309
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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