RETROGRADE CEREBRAL PERFUSION PROVIDES LIMITED DISTRIBUTION OF BLOOD TO THE BRAIN: A STUDY IN PIGS

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Objective: The objective of this study was to investigate flow distribution during retrograde and antegrade cerebral perfusion with India ink as a marker. **Methods:** Ten pigs received cerebral perfusion with a solution containing 50% filtered India ink for 5 minutes either antegradely through both internal carotid arteries at a flow of 180 to 200 ml/min ($n = 5$) or retrogradely via the superior vena cava at a flow of 300 to 500 ml/min ($n = 5$). The brains were then fixed for quantitative measurement of the density of ink-filled capillaries (reported as a percentage of the total selected area). The assessment was done with the use of an in-house software program. **Results:** In the antegrade cerebral perfusion group, the intracranial arterial and venous systems were completely filled with ink. The gray matter was colored uniformly black, and light coloring was observed in the white matter. During retrograde cerebral perfusion, the majority of ink was returned to the inferior vena cava, and only a small amount of ink was found in the innominate artery draining from the brain. Massive ink filling was observed in the sagittal sinus and other venous sinuses in all the pigs. Vessels on the surface of the brain and large vessels in the brain were also well filled with ink. However, only 10% of capillaries were filled with ink during retrograde cerebral perfusion relative to the number observed with antegrade cerebral perfusion. **Conclusions:** Retrograde cerebral perfusion supplies a limited amount of blood to brain tissue, which flows mainly through superficial and large deep cerebral vessels. (J Thorac Cardiovasc Surg 1997;114:660-5)

Different methods are used to reduce brain damage during aortic arch operations such as for dissecting aneurysms of the ascending aorta and arch or for congenital heart disease in infancy. Recent techniques include deep hypothermic circulatory arrest in association with retrograde cerebral perfusion via the superior vena cava.^{1,2} This method is gaining popularity and reduces neurologic dam-

age after aortic arch surgery.¹⁻⁴ However, neurologic morbidity after deep hypothermic circulatory arrest is widely recognized, especially after prolonged periods of circulatory arrest (longer than 45 to 60 minutes).⁵⁻⁷ Previous studies from our laboratory demonstrate that retrograde cerebral perfusion via the superior vena cava provides only limited brain protection during prolonged deep hypothermic circulatory arrest (120 minutes at 15° C) in the pig.^{7,8} It has been suggested that the majority of blood is shunted into the body rather than to the brain.⁹ However, the distribution of blood inside the brain during retrograde cerebral perfusion remains unknown. The present study was designed to investigate flow distribution to the brain during retrograde cerebral perfusion with India ink as a marker.

Material and methods

All animals received humane care in compliance with the guidelines of the Canadian Council on Animal Care.

Animal preparation. Ten neurologically mature¹⁰ male and female young pigs (commercial farm Yorkshire cross

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Fig. 1. Ink-filled vessels on the surface of the brain. **A,** Antegrade cerebral perfusion group. **B,** Retrograde cerebral perfusion group.

bred, 20 to 28 kg, 84 to 97 days old) were used. Preanesthesia was induced with xylazine (2.2 mg/kg), ketamine (20 mg/kg) and atropine (0.03 mg/kg) injected intramuscularly. After endotracheal intubation, the animal's lungs were ventilated mechanically with 60% oxygen mixed with air. The ventilator rate and tidal volume were adjusted to maintain the arterial carbon dioxide tension in the normal range. Anesthesia was maintained with 1.0% to 2.0% isoflurane. The chest was opened via a median sternotomy. Heparin was given intravenously at a rate of 300 IU/kg. A venous cannula was placed into the superior vena cava to provide retrograde cerebral perfusion in the retrograde cerebral perfusion group. Two identical cannulas were inserted into the internal carotid arteries and were connected to the perfusion line via a Y connector to provide antegrade perfusion of the brain in the antegrade cerebral perfusion group. Perfusion pressure was monitored during both antegrade and retrograde cerebral perfusion. A Cobe roller bypass pump (model C22.2; Cobe Laboratories, Inc., Lakewood, Colo.) was used for ink perfusion of the brain. India ink (Royal, Reeves & Poole Group Inc., Downsview, Ontario, Canada) was diluted to 50% with normal saline solution and filtered through a 20 μ m arterial filter.

The pigs were randomly assigned to one of the two groups: group 1, retrograde cerebral perfusion ($n = 5$); group 2, antegrade cerebral perfusion ($n = 5$). In both groups, the heart was arrested with the use of a high-potassium solution and the pig was exsanguinated by severing the descending aorta. Immediately after exsanguination, the brain was perfused antegradely or retrogradely with ink solution at room temperature (20° to 21° C) for 5 minutes. In the antegrade perfusion group, brain perfusion was established via both carotid arteries, and the returned ink was drained by opening the inferior and superior venae cavae. As described previously,⁷ the perfusion pressure was maintained at 65 to 85 mm Hg, with a concomitant flow of 180 to 200 ml/min. In the retrograde group, the brain was continuously perfused with ink through the superior vena cava; drainage of the returned ink was established through both the aorta and the inferior vena cava and carefully observed. The azygos vein was tied in this group. Perfusion pressure at the

superior vena cava was maintained at 35 to 45 mm Hg, with a concomitant flow of 300 to 500 ml/min.

Tissue preparation. After the ink perfusion had been completed, the intracranial vessel systems were carefully observed, and the brain was removed and immersed in a 10% buffered formaldehyde solution at 4° C for 2 weeks. The brain was separated into anatomic regions of interest, including cortex (frontal, cingulate, and temporal), caudate nucleus, putamen, thalamus, cerebellum, pons, hippocampus, and mesencephalic gray. The tissue blocks were further cut into approximately 1 $\times$ 1 $\times$ 0.5 cm slabs (samples), which were transferred to a 30% sucrose solution and kept at 4° C overnight. The tissue slabs were placed into a 2-methylbutane solution (–40° to –50° C) for 30 to 60 seconds and were then transferred to a cryostat (–40° C) for 5 minutes. The samples were stored at –70° C. The prepared samples were cut into 20 μ m thick slices with a cryostat (at a temperature of –25 to –28° C). The slices were mounted on uncoated slides and dried at room temperature.⁷

Quantification of density of ink-filled capillaries. The quantitative density of ink-filled capillaries in a selected area was measured under 100-fold magnification in five slides from every tenth section that contained the same brain region with the use of Line_Measure, an in-house, interactive computer program used for measuring the fraction of an image covered by lines having a width within a specified range. The program was developed with the IDL programming language. After a threshold brightness level has been set, the chosen image is converted to a black-and-white image that distinguishes stained from unstained tissue. Minimum and maximum widths measured in pixels are specified for the lines of interest. They are then refined interactively, allowing the user to set the values that identify the ink-stained capillaries, but they exclude smaller "speckle noise" and larger vessels in the image. Line_Measure then applies the "erode" and "dilate" operations of mathematical morphology¹¹ to identify the regions of the image that belong to lines within the specified range of widths. The value of the density of the ink-filled capillaries is expressed as a percentage of the total selected area.

Fig. 2. Coronal sections of the brain. **A**, Antegrade cerebral perfusion group. **B**, Retrograde cerebral perfusion group.

The average density of ink-filled capillaries in each region was then calculated from five measurements. Vessels with a diameter less than 10 μm were counted as capillaries. All other ink-filled vessels were arbitrarily treated as large vessels in this particular situation, because it was not necessary to further define these vessels. The pathologist was blinded to group assignment.

Statistical analysis. All data are presented as mean $\pm$ standard error of the mean. All the statistical analyses were performed with the use of Statistica (Statsoft, Tulsa, Okla.). Comparisons between groups were carried out with the use of the Mann-Whitney U test and Student's *t* test with the Bonferroni adjustment for multiple comparisons.^{12, 13} A Kruskal-Wallis analysis of variance by ranks was used for comparisons between the different regions of the brain (ten regions) within a group. Both 95% and 99% simultaneous confidence intervals for the mean difference^{13, 14} are also reported.

Results

In the antegrade cerebral perfusion group, ink returned from the brain mainly via the superior vena cava and a small amount of the ink drained through the inferior vena cava. The head, tongue, neck, and part of the frontal limbs were completely colored black. Opening the skull revealed that the intracranial arterial and venous systems were completely filled with ink (Fig. 1, *A*). Coronal sections of the brain showed that the gray matter was uniformly colored black; light coloring was observed in the white matter (Fig. 2, *A*). The density of ink-filled capillaries in ten brain regions is shown in Table I. No difference in density of ink-filled capillaries was found among the ten brain regions.

During retrograde cerebral perfusion, both inter-

nal jugular veins were completely filled with ink, and no blocking valve was seen in these veins. The majority of ink returned via the inferior vena cava, and only a small amount of ink was found in the innominate artery that collected from the brain. Coloring of the head, tongue, neck, and frontal limbs was less than in the antegrade cerebral perfusion group. Massive ink filling was observed in the sagittal sinus and other venous sinuses in all the pigs. The vessels on the surface of the brain were also well filled (Fig. 1, *B*). In coronal sections of the brain, the degree of coloring in both the gray and the white matter was markedly lower than that observed in the antegrade perfusion group (Fig. 2). The superficial areas of the gray matter (about 1 mm from the surface) showed more coloring than the deeper areas. On microscopic examination, the large vessels in the brain were well filled with ink. However, the density of the ink-filled capillaries in ten regions of the brain was significantly less than in the antegrade cerebral perfusion group (Tables I and II, Fig. 3). No significant difference among the ten regions was found in this group.

Discussion

This study was designed to determine the distribution of flow to the brain during retrograde cerebral perfusion via the superior vena cava in pigs. Five minutes of cerebral perfusion was chosen to ensure flow through the entire brain while avoiding accumulation of large amounts of ink in the tissue, which occurs after prolonged perfusion.

This study demonstrates, not unexpectedly, that antegrade cerebral perfusion provides complete and uniform distribution of dye to the brain. This is in agreement with our previous findings that antegrade cerebral perfusion preserves normal energy metabolism,⁸ neuronal morphology,⁷ and membrane-associated protein 2 immunoreactivity¹⁵ during prolonged deep hypothermic circulatory arrest (120 minutes at 15°C). The present results show that retrograde cerebral perfusion via the superior vena cava supplies limited dye to brain tissue and that a large amount of dye is shunted into the inferior vena cava, even though no noticeable valvular obstruction was present in the internal jugular veins. Anatomic observations on the internal jugular veins in the pigs used for our studies revealed that, in most of the pigs, there was no valve or only one incomplete valve at the origin of the internal jugular vein. In most cases, this valve did not cause significant blockage of blood flow. The massive amount of ink returned from the inferior vena cava

Table I. Density of ink-filled capillaries in ten regions of the brain

	Antegrade cerebral perfusion	Retrograde cerebral perfusion	<i>p</i> Value*
Frontal cortex	3.00 ± 0.28	0.42 ± 0.07	0.01
Cingulate cortex	2.70 ± 0.13	0.29 ± 0.10	0.01
Temporal cortex	3.03 ± 0.27	0.30 ± 0.09	0.01
Caudate nucleus	2.97 ± 0.54	0.35 ± 0.19	0.01
Putamen	2.64 ± 0.60	0.23 ± 0.12	0.01
Thalamus	2.71 ± 0.24	0.77 ± 0.15	0.01
Cerebellum (granular layer)	3.31 ± 0.52	0.33 ± 0.09	0.01
Pons	2.53 ± 0.31	0.68 ± 0.15	0.01
Mesencephalic gray	2.63 ± 0.34	0.39 ± 0.14	0.01
Hippocampus	2.44 ± 0.25	0.36 ± 0.13	0.01

All the values shown as percentage of the total selected area (mean ± standard error of the mean).

*The Mann-Whitney U test.

observed in our study is consistent with the findings of Boeckxstaens and Flameng¹⁶ in baboons. The small amount of blood that reaches the brain is mainly circulated in the intracranial venous sinuses and the superficial veins of the brain. The large vessels inside the brain were also well filled with ink. Only about 10% of the capillaries in the brain were perfused during retrograde cerebral perfusion relative to the number observed with antegrade perfusion. This is probably due to abundant venovenous communication with the high-capacity, low-resistance inferior vena caval bed. A review of the literature shows that only 1% to 20% of blood perfused in the superior vena cava returns from the innominate and left carotid arteries.^{9, 16, 17}

The findings from this study explain previous results that retrograde perfusion during deep hypothermic circulatory arrest provides better cerebral protection than deep hypothermic circulatory arrest alone, but less brain protection relative to antegrade cerebral perfusion during prolonged (120 minutes) deep hypothermic circulatory arrest.^{7, 8} Limited perfusion of the brain during retrograde cerebral perfusion plays an important role in reducing brain damage by keeping the brain cool, removing metabolites and emboli, and providing nutrients during circulatory arrest. Clinical studies have shown that retrograde cerebral perfusion extends the "safe" time for hypothermic circulatory arrest with reduced risk of adverse neurologic events.¹⁸⁻²¹ However, retrograde cerebral perfusion has also been shown to be unable to provide as good protection to the brain as does antegrade cerebral perfusion during prolonged hypothermic circulatory arrest.^{7, 8, 22}

Fig. 3. Ink-filled vessels in brain tissue (original magnification $\times 100$). **A**, Antegrade cerebral perfusion group. **B**, Retrograde cerebral perfusion group.

Table II. Confidence intervals and statistical results with the Bonferroni adjustment

Brain areas	Mean difference (Ant.-Ret.)	p Values*	95% CI†	99% CI†
Frontal cortex	2.583	0.0009	[1.216, 3.950]	[0.779, 4.388]
Cingulate cortex	2.406	0.00004	[1.774, 3.039]	[1.571, 3.241]
Temporal cortex	2.732	0.0005	[1.634, 3.829]	[1.283, 4.180]
Caudate nucleus	2.611	0.06	[0.423, 4.799]	[-0.277, 5.499]
Putamen	2.409	0.1	[0.075, 4.744]	[-0.672, 5.491]
Thalamus	1.949	0.004	[0.885, 3.014]	[0.544, 3.354]
Cerebellum	2.984	0.01	[0.982, 4.986]	[0.342, 5.627]
Pons	1.852	0.02	[0.546, 3.157]	[0.128, 3.575]
Mesencephalic gray	1.930	0.009	[0.404, 3.455]	[-0.126, 3.985]
Hippocampus	2.076	0.003	[0.993, 3.159]	[0.647, 3.506]

Ant.-Ret., Antegrade-retrograde.

*Student's *t* test (antegrade vs retrograde) with the Bonferroni adjustment.

†Simultaneous confidence intervals (CI) for the mean difference.

Limited blood delivery to the brain during retrograde cerebral perfusion is one of the major factors affecting brain protection. On the basis of our previous magnetic resonance spectroscopy study in the same model, the small amount of blood flow that is provided by retrograde cerebral perfusion during hypothermic circulatory arrest is unable to prevent ischemic metabolism, as indicated by a significant decrease of adenosine triphosphate and creatine phosphate and accumulation of inorganic phosphate.⁸ However, the lowest blood flow required to avoid anaerobic metabolism and to maintain normal cerebral function during retrograde cerebral perfusion remains unknown.

Because of a possible difference in viscosity between India ink solution and hemodiluted whole blood, the flow distribution of hemodiluted whole blood may be more effective than that of India ink solution during retrograde cerebral perfusion. 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 are often not feasible in human beings. Data from our studies^{7,8} further our understanding of the consequences of retrograde cerebral perfusion on flow distribution, pH, metabolism, and morphology in the brain and suggest procedures that could improve brain protection during cardiac operations.

In conclusion, retrograde cerebral perfusion via the superior vena cava in pigs results in significantly less uniform flow distribution to the brain than does antegrade cerebral perfusion. The optimal conditions and routes for improving blood distribution to brain tissue during retrograde cerebral perfusion remain to be determined.

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