Cerebral Ischemia and Reperfusion Increases the Heterogeneity of Local Oxygen Supply/Consumption Balance

Harvey R. Weiss, PhD; Jeremy Grayson, MD; Xia Liu, MD; Sylviana Barsoum, MD; Harsh Shah; Oak Z. Chi, MD

Background and Purpose—After cerebral vessel blockage, local blood flow and O₂ consumption becomes lower and oxygen extraction increases. With reperfusion, blood flow is partially restored. We examined the effects of ischemia-reperfusion on the heterogeneity of local venous oxygen saturation in rats in order to determine the pattern of microregional O₂ supply/consumption balance in reperfusion.

Methods—The middle cerebral artery was blocked for 1 hour using the internal carotid approach in 1 group (n=9) and was then reperfused for 2 hours in another group (n=9) of isoflurane-anesthetized rats. Regional cerebral blood flow was determined using a C¹⁴-iodoantipyrine autoradiographic technique. Regional small vessel arterial and venous oxygen saturations were determined microspectrophotometrically.

Results—After 1 hour of ischemia, local cerebral blood flow (92±10 versus 50±10 mL/min per 100 g) and O₂ consumption (4.5±0.6 versus 2.7±0.5 mL O₂/min per 100 g) decreased compared with the contralateral cortex. Oxygen extraction increased (4.7±0.2 versus 5.4±0.3 mL O₂/100 mL) and the variation in small vein (20–60 μm) O₂ saturation as determined by its coefficient of variation (=100×SD/mean) increased (5.5 versus 10.5). With 2 hours of reperfusion, the blood flow decrement was reduced and O₂ consumption returned to the value in the contralateral cortex. Oxygen extraction remained elevated in the ischemic-reperfused area and the coefficient of variation of small vein O₂ saturation increased further (17.3).

Conclusions—These data indicated continued reduction of O₂ supply/consumption balance with reperfusion. They also demonstrated many small regions of low oxygenation within the reperfused cortical region. (Stroke. 2013;44:2553-2558.)

Key Words: cerebral blood flow ■ cerebral O₂ consumption ■ cerebral O₂ supply/consumption balance ■ ischemia-reperfusion

When a cerebral artery is blocked, there is a decrease in local blood flow.¹,² Despite a rise in regional O₂ extraction, local O₂ consumption decreases in the ischemic brain region.³ Glucose usage is significantly reduced.⁴ During ischemia, cerebral oxygenation also decreases.⁵,⁶ This is accompanied by a fall in local-tissue oxygen tension and the oxygen saturation of the small veins draining the ischemic area.⁵,⁷ Capillary perfusion, tissue oxygenation, and O₂ supply/consumption balance are heterogeneous under both normal and ischemic conditions in the brain.⁵,⁸-¹⁰ There is good evidence for a heterogeneous distribution of brain injury during continued ischemia.¹¹ It is not clear how all of these parameters and their heterogeneity are changed on reperfusion.

The primary treatment for ischemic stroke is recanalization therapies (or thrombolitics).¹²,¹³ This treatment helps to restore regional cerebral blood flow (CBF) toward normal in the ischemic area.¹⁴,¹⁵ Prompt treatment is usually beneficial and reduces the degree of ischemic damage. The return of blood flow to the ischemic region may or may not restore oxygen consumption and glucose usage.¹⁶,¹⁷ Middle cerebral artery occlusion and reperfusion in the rat provide a good model for brain ischemia-reperfusion studies.¹⁸,¹⁹ There is evidence for an increase in tissue oxygenation in the reperfused region in both rats and human.²⁰ It is not clear whether this restoration of flow after a period of cerebral ischemia uniformly or heterogeneously affects regional O₂ supply/consumption balance.

In this study, we tested the hypothesis that reperfusion would improve O₂ supply/consumption balance but that the improvement would not be homogeneous. We had previously demonstrated heterogeneity during ischemia.²¹ This was examined in isoflurane-anesthetized rats subjected to middle cerebral artery occlusion and reperfusion. We measured cerebral flow and arterial and venous oxygen saturations. We found reduced flow and oxygen consumption and increased O₂ extraction with occlusion. With reperfusion, the flow decrement was reduced and oxygen consumption was returned toward control.

Received February 12, 2013; final revision received April 29, 2013; accepted May 22, 2013.

From the Department of Neuroscience and Cell Biology (H.R.W., H.S.) and Department of Anesthesiology (J.G., X.L., S.B., O.Z.C.), Rutgers Robert Wood Johnson Medical School, Piscataway, NJ.

The online-only Data Supplement is available with this article at http://stroke.ahajournals.org/lookup/suppl/doi:10.1161/STROKEAHA.113.001172/-/DC1.

Correspondence to Harvey R. Weiss, PhD, Department of Neuroscience and Cell Biology, Rutgers Robert Wood Johnson Medical School, 675 Hoes Lane W, Piscataway, NJ 08854, E-mail hweiss@rwjms.rutgers.edu

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Stroke is available at http://stroke.ahajournals.org DOI: 10.1161/STROKEAHA.113.001172

2553
levels, but oxygen extraction remained elevated. The O₂ supply/consumption balance became more heterogeneous during occlusion and the heterogeneity increased on reperfusion.

Methods

This investigation was conducted in accordance with US Public Health Service Guidelines using the Guide for the Care of Laboratory Animals (DHHS Publication No. 85-23, revised 1996) and was approved by our Institutional Animal Care and Use Committee. Eighteen male Fischer 344 rats (3 months) were divided into cerebral ischemic (n=9) and reperfusion (n=9) groups. Each rat was used to measure regional CBF and microscopic arterial and venous oxygen saturations. The rats were initially anesthetized with 2% isoflurane in an air and oxygen mixture through a tracheal tube to maintain the arterial P₀₂ at >100 mm Hg. A femoral artery and vein were cannulated. The venous catheter was used to administer radioactive tracer. The artery catheter was connected to a pressure transducer and an InVivos data acquisition system was used to monitor heart rate and blood pressure. This catheter was also used to obtain arterial blood samples for analysis of hemoglobin, blood gases, and pH using a Radiometer blood gas analyzer. The isoflurane concentration was decreased to 1.4%. Body temperature was monitored and maintained at 37°C with a servo-controlled rectal thermistor probe and a heating lamp.

We used the transient occlusion of the middle cerebral artery (MCA) using an intraluminal thread as our technique to study cerebral ischemia-reperfusion. The common carotid artery was exposed through a midline ventral cervical incision and carefully separated from the adjacent nerve. Then, a 4.0-monofilament thread with its rounded tip was inserted into the stump of the external carotid artery and advanced ≈1.7 cm into the internal carotid artery until resistance was felt. The filament was held in place for 60 minutes blocking the MCA in the ischemic group. In the reperfusion group, the filament was then removed, allowing reperfusion, and the external carotid artery was closed. Measurements were performed after 120 minutes of reperfusion. Regional CBF and microscopic O₂ saturations of small veins and arteries were determined in several brain regions in both groups of animals.

Regional CBF was measured by the 14C-iodoantipyrine quantitative autoradiographic technique. Briefly, 40-μCi of 14C-iodoantipyrine was infused intravenously. When the isotope entered the venous circulation, the arterial catheter was cut to 20 mm to minimize smearing. Twenty-μl blood samples were obtained from the arterial catheter approximately every 3 s during next 60 s. At the moment when the last sample was obtained, the animal was decapitated and the head was frozen in liquid nitrogen. After freezing, the brain was sampled from 3 regions: ischemic cortex, contralateral cortex, and pons. The brain samples were sectioned (20 μm) on a microtome-cryostat and the sections were exposed to x-ray film to obtain an autoradiogram. The cerebral 14C-iodoantipyrine concentrations were determined by reference to recalibrated standards using the NIH imageJ program. For each brain region examined, a minimum of 8-optical density measurements were made, each on different sections. Blood samples were placed in a tissue solubilizer and 24 hours later, put in a counting liquid. These samples were counted on a liquid scintillation counter and were quench-corrected. Regional CBFs were then calculated.

Alternate sections from regions of the same brain (ischemic cortex, contralateral cortex, and pons) were used for the determination of arterial and venous O₂ saturation. The cortical regions were from a 5-mm plug from the parietal cortex over the MCA. Details of this technique have been published previously. Briefly, the brain sections were cut into wafers at −20°C. Twenty-μ thick sections were obtained at −35°C under a N₂ atmosphere. The sections were transferred to precooled glass slides and covered with degassed silicone oil and a coverslip. These slides were placed on a microspectrophotometer fitted with an N₂-flushed cold stage to obtain readings of optical density at 568, 523, and 560 nm. This 3-wavelength method corrects for the light scattering in the frozen blood. Only vessels cut in transverse section were studied, so that the path of light traversed only the blood. The size of the measuring spot was 8 μm in diameter. Readings were obtained to determine O₂ saturation in 5 arteries and 10 veins (20–60 μm in diameter) in each region. The O₂ content of blood was determined by multiplying the percentage of O₂ saturation by the hemoglobin concentration times 1.36, the maximal binding capacity of hemoglobin for O₂ per gram. The difference between the average arterial and venous O₂ contents (regional O₂ extraction) was then obtained. Using the Fick principle, we calculated the O₂ consumption for each region as the product of average flow and O₂ extraction. This method has been validated in the brain.

Analysis of variance using a repeated measure design was performed for the various measurements to assess the difference between the different regions and treatments examined for hemodynamic, blood gases, CBF, O₂ extraction, and O₂ consumption values. Post hoc testing of multiple comparisons was performed using Tukey’s procedure. The coefficient of variation of venous oxygen saturations (SvO₂) was used to compare changes in heterogeneity. The coefficient of variation was calculated as 100×SD/mean. A χ² test was used to assess differences in the distribution of SvO₂ and differences in the number of low O₂ saturated veins between groups. A value of P<0.05 was considered as statistically significant. All values are expressed as mean±SEM.

Results

Hemodynamic and blood gas parameters in the ischemic (1 hour MCA occlusion) and (1 hour MCA occlusion+2 hours reperfusion) reperfusion groups of rats were within the normal ranges for anesthetized rats (Table). There were no statistically significant differences in arterial blood pressures between groups. Heart rates were also similar between the 2 groups of rats. Arterial blood gases and pH were controlled and also were not significantly different between the 2 experimental groups.

CBF in the ischemic cortex was significantly reduced compared with the contralateral cortex in the ischemic group Figure 1. The decrement was to 54% of the value found in the contralateral cortex. In the reperfusion group, flow in the ischemic region returned toward the value in the contralateral cortex. The difference in flow between the ischemic and contralateral cortical regions was not statistically significant in the reperfusion group. Blood flow in the ischemic region was significantly higher in the reperfused group compared with the ischemic group.

The oxygen extraction of the ischemic cortex was significantly elevated (+15%) compared with the contralateral cortex in the ischemic group Figure 2. Similarly, the oxygen extraction was also significantly elevated in the reperfusion group in the ischemic-reperfused region (+21%) compared with the

| Table. Hemodynamic and Blood Gas Values for the Ischemic and Reperfusion Groups |
|-----------------|-----------------|
| Systolic blood pressure, mm Hg | 127±9 | 118±10 |
| Diastolic blood pressure, mm Hg | 89±9 | 84±10 |
| Mean blood pressure, mm Hg | 101±9 | 95±10 |
| Heart rate, beats per min | 296±17 | 290±17 |
| Arterial P₀₂, mm Hg | 131±6 | 123±9 |
| Arterial pH | 7.36±0.02 | 7.37±0.05 |

Values are mean±SEM. n=9 per group.
contralateral cortex. There were no differences in O$_2$ extraction in either the ischemic or contralateral cortex in comparison between the control and the reperfusion groups Figure 2.

Arterial O$_2$ saturations were similar in all regions and groups (Table II in the online-only Data Supplement). However, venous O$_2$ saturation was significantly lower in the ischemic or ischemic-reperfused regions compared with the contralateral cortex in both the ischemic and reperfused groups. The distribution of small vein (20–60 μm diameter) O$_2$ saturations was heterogeneous in all of these cortical regions Figure 3. There was a shift to lower values in the ischemic and ischemic-reperfused cortex compared with the contralateral cortex. The O$_2$ saturation distribution was more homogeneous in the reperfusion group. The number of vessels with O$_2$ saturations <50% was significantly greater in the ischemic-reperfused cortex compared with the contralateral cortex in the ischemic group. The coefficient of variation increased further in the ischemic-reperfused region of the reperfusion group (17.3). Examples of some of these small cerebral veins are shown in Figure 4.

Cerebral O$_2$ consumption in the ischemic cortex was significantly reduced compared with the contralateral cortex in the ischemic group Figure 5. This decrement was to 60% of the value found in the contralateral cortex. In the reperfusion group, cerebral O$_2$ consumption in the ischemic region was similar to the value found in the contralateral cortex. Cerebral O$_2$ consumption in the ischemic region was significantly higher in the reperfused group compared with that found in the ischemic group.

We also compared the effects of ischemia and ischemia-reperfusion in a remote cerebral region, the pons. There were no significant differences in the CBF (138±26 mL/min per 100 g ischemic versus 119±14 reperfusion) in the pons between groups. The oxygen extraction (4.7±0.2 mL O$_2$/100 mL ischemic versus 4.5±0.2 reperfusion) was also similar between the 2 experimental groups in the pons. The oxygen consumption of the pons was also not significantly different in comparison between the ischemic and reperfusion groups (6.7±1.5 mL O$_2$/min per 100 g ischemic versus 5.7±0.7 reperfusion).

**Discussion**

Under control conditions, there was heterogeneity of small cerebral vein O$_2$ saturations. This heterogeneity increased and the average value decreased during cerebral ischemia. Blockage of the MCA was also associated with a fall in CBF and O$_2$ consumption. The major findings of this study were that with reperfusion, blood flow was partially restored and O$_2$ consumption returned to the value found in the contralateral cortex. Small cerebral vein O$_2$ saturations remained low and their heterogeneity increased above that found with ischemia alone. This indicated many small regions of low oxygenation within the reperfused cortical region.

Roy and Sherrington$^{23}$ first claimed that changes in CBF were precisely regulated to meet the requirements of cerebral metabolism. On the level of the whole brain, this close linkage between metabolism and blood flow has been shown in a variety of conditions.$^{24}$ A consequence of the linkage between cerebral flow and metabolism is that global (sagittal sinus) cerebral O$_2$ saturation and cerebral O$_2$ extraction do not usually change. If microregional CBF precisely matched local cerebral O$_2$ consumption, there should be no differences in local-tissue P$_{O_2}$, local venous O$_2$ saturation, or O$_2$ extraction within various small regions of the brain. From measurements of cerebral oxygenation, tissue P$_{O_2}$ and O$_2$ saturation from small veins, it is clear that significant heterogeneity exists.$^{8,25,26}$ We found variation in basal small vein O$_2$ saturations, which are draining local areas, under control conditions. The regional ratio of O$_2$ supply to consumption is determined by dividing local O$_2$ supply by local O$_2$ consumption, Cao$_2$ · CBF/CBF · (Cao$_2$–CvO$_2$), where Cao$_2$ and CvO$_2$ are the arterial and venous O$_2$ content and CBF is the cerebral flow. This reduces to SaO$_2$/Sao$_2$–SvO$_2$, where SaO$_2$ and SvO$_2$ are the percentages of oxyhemoglobin in the arterial and venous blood, respectively. Because SaO$_2$ is a relative constant, SvO$_2$ variability represents the O$_2$ supply/consumption heterogeneity, which varies at baseline. The coefficient of variation of
SvO₂ was 5.5 in the contralateral cortex in the ischemic group and 5.7 in the reperfusion group. This is a good measure of O₂ supply/consumption balance and similar to previous reports from our group in the normal cortex.¹⁷,²⁵

With blockage of the MCA, local blood flow and O₂ consumption decline.¹ Glucose usage is also depressed in cerebral ischemia.¹⁶ Cerebral oxygen extraction increased and SvO₂ declined in the affected region. This has been observed in both cats and rats.³,²⁷ Tissue Po₂ also significantly declines.³ Deoxyhemoglobin levels may be increased in humans with stroke.²⁸ Without further treatment, parts of this area will have both loss of function and irreversible damage.¹,¹⁸

From observation of Figure 3, it is clear that only some vessels show a lower O₂ saturation in the occluded region. This leads to a downward shift in SvO₂ and an increase in heterogeneity in the occluded region, although we cannot link this heterogeneity directly to either the core or penumbra regions. The coefficient of variation of SvO₂ was 10.5 in the ischemic cortex of the ischemic group. This had been observed previously with cerebral ischemia.¹⁰,²⁷ Some of this heterogeneity may be because of heterogeneity of local capillary transit times or perfusion patterns.¹⁰ The full control of this heterogeneity is not yet known. These data indicate many microregions with an impaired O₂ supply/consumption balance, but other regions where this balance is essentially normal. This may lead to significant heterogeneity of damage in the ischemic region.¹¹

With removal of the filament, blood flow returned toward control values in the ischemic-reperfused region. This is similar to other reports.¹²,¹⁸ Oxygen consumption in the reperfused brain region also returned toward values found in the contralateral cortex. We had previously reported a fall in local O₂ consumption with cerebral vessel blockage,³,²⁷ although this study seems to be the first quantitative measurement of its return during reperfusion. Others have reported some return of oxygen consumption or glucose uptake or a continued depressed usage.¹⁶,¹⁷,²⁹ However, local cerebral oxygen extraction remained elevated and venous O₂ saturations remain low in the ischemic-reperfused region. Tissue oxygen tension may or may not return to normal during reperfusion.⁵,²⁰ Our data indicate some, but not complete recovery in the previously ischemic area.

Venous oxygen saturation in the reperfused cortex was depressed compared with the contralateral cortex and not significantly different from that in the ischemic group. Examination of the histogram of SvO₂ emphasized the fact that only some of the small veins drained regions extracting

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**Figure 3.** Distribution of O₂ saturations (%) of small veins in the ischemic cortex (top) and the ischemic-reperfused cortex of the reperfusion group (bottom). Note that the shift to lower values in the ischemic and ischemic reperfused cortex compared with the contralateral cortex.
Weiss et al
Reperfusion and Cerebral O₂ Supply/Consumption

more oxygen. This led to a significant increase in heterogeneity for SvO₂. The coefficient of variation of SvO₂ rose to 17.3. Thus, the O₂ supply/consumption balance within the reperfused region is quite variable. Some microregions have a reduced balance, whereas in others, it is back to control levels. This could be related to a reduced cerebral capillary flow velocity and increased heterogeneity of this flow during reperfusion.³⁰ Restoring capillary flow either directly or by reducing inflammation in these flow restricted microregions might prove beneficial during reperfusion.³¹,³² There is evidence for a heterogeneous distribution of brain injury during ischemia and reperfusion.¹¹ This heterogeneous pattern of cerebral O₂ supply/consumption balance during reperfusion might be related to the initial ischemic damage or to reperfusion injury.³³ This seems to be the first study to demonstrate a heterogeneous return of cerebral O₂ supply/consumption balance during reperfusion. Blood flow and O₂ consumption have returned toward control levels in the reperfused region. However, microregional cerebral O₂ supply/consumption balance shows many regions drained by small cerebral veins with high oxygen extraction. This is similar to reports during occlusion.³⁴ These regions are alive because they are using oxygen. However, it is not clear whether they will die later. It is also possible that some of the regions with normal venous oxygen saturations have lost neuronal tissue. This heterogeneous pattern of cerebral O₂ supply/consumption balance during reperfusion suggests that further treatments may lead to a reduction in ischemic damage. Because only some small veins have low oxygenation, this could be related to microvascular blockage or changes in capillary flow heterogeneity. Reducing this heterogeneity might help. Alternatively, reducing the oxygen demand in these low oxygenation regions could also preserve tissue. Future work is necessary to examine these possibilities.

In summary, blockade of the middle cerebral artery led to a fall in regional flow and O₂ consumption. This was associated with an increased cerebral O₂ extraction and heterogeneity of local venous oxygen saturation. The major findings of this study were that with reperfusion of the middle cerebral artery, blood flow was partially restored and O₂ consumption returned to the value found in the contralateral cortex. Small vein O₂ saturations remained low and their heterogeneity increased above that found with ischemia alone. This indicated many microregions of low oxygenation within this reperfused cortical area.

Disclosures
None.

References
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Stroke. 2013;44:2553-2558; originally published online July 9, 2013;
doi: 10.1161/STROKEAHA.113.001172

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SUPPLEMENTAL MATERIAL

Table II Arterial and venous O2 saturations (%) in the ischemic or ischemic-reperfused and contralateral cortex of the ischemic and reperfusion groups

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Values are mean±S.E.M. (N=9 per group) * Significantly different from contralateral cortex