Transient Middle Cerebral Artery Occlusion Influence on Systemic Oxygen Homeostasis and Erythropoiesis in Wistar Rats

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Background and Purpose—Systemic hypoxia is a common complication in stroke patients and may exacerbate ischemic brain damage. Expression of the hypoxia-inducible cytokine erythropoietin (Epo) is upregulated in the brain in both stroke patients and in animal stroke models and exerts local neuroprotective effects in the ischemic brain. Epo is also well known to stimulate red blood cell (RBC) production. The purpose of the present study was to evaluate whether poststroke systemic hypoxia is present in the rat model and whether it is associated with increased peripheral Epo and RBC production.

Methods—Wistar rats underwent 1-hour transient middle cerebral artery occlusion (MCAO) under mechanical ventilation, followed by reperfusion without further ventilation. Groups of MCAO and sham-operated animals were evaluated at extended times after reperfusion for assessment of arterial blood gases, plasma Epo, and complete blood count.

Results—Arterial oxygen saturation was significantly lower in the infarct group between 6 and 24 hours after reperfusion (P<0.0005), and plasma Epo levels were increased 6 hours after reperfusion (P<0.05). RBC counts and hematocrit were transiently increased 2 to 7 days after reperfusion in animals with MCAO compared with sham. Maximal increases were seen at day 7 (22% and 16% increases of RBC count and hematocrit, respectively; P<0.001). In contrast, the white cell blood counts in animals with MCAO decreased by >30% in the same time period.

Conclusions—Plasma Epo levels, RBC counts, and hematocrit are all increased in response to systemic hypoxia after cerebral ischemia in rats. (Stroke. 2004;35:1979-1984.)

Key Words: hypoxia ■ hematocrit ■ stroke
A study was to evaluate the changes in plasma Epo, RBC numbers, and hematocrit from days 0 to 28 after transient MCAO in Wistar rats and to examine whether these changes are associated with systemic hypoxia.

Materials and Methods

Animals

All procedures were performed in accordance with current guidelines of the Canadian Council on Animal Care. Adult male Wistar rats (Charles River Breeding Center, St Constant, Quebec, Canada) were housed in groups of 3 to 5 and maintained under a natural 14/10-hour light/dark cycle, with food and water available ad libitum. Before surgery, animals were fasted for 2 hours and then weighed once before surgery and again at the moment of euthanization. Animal weights were uniform at the beginning of the experiments and ranged between 350 and 400 g.

Transient MCAO

The transient MCAO technique was modified from Zhu and Auer, as described previously. Anesthesia was induced with 5% isoflurane inhalation and maintained with 1% to 2% isoflurane in nitrous oxide/oxygen (70%/30%). Animals were intubated and ventilated (model 683 rodent ventilator; Harvard). Rectal temperature was maintained throughout surgery at 37°C with a heating blanket (homeothermic blanket control unit; Harvard). The tail artery was cannulated for blood sample collection and blood pressure monitoring (Gould 13-6615-50 preamplifier and 6600 amplifier, Mark8 recorder; Graphtec). Blood gases and glucose level before and immediately after ischemia were obtained. The total amount of blood removed never exceeded 400 mL and was replaced with an equal volume of saline. Arterial blood gases (PaO₂, PaCO₂, SaO₂, pH) were measured using a blood gas analyzer (ABL520; Radiometer). Animal ventilatory rate was adjusted to maintain normal levels of oxygen and carbon dioxide. MCAO was induced with a 3-0 monofilament coated with poly-L-lysine at a mean arterial blood pressure (MABP) of 60 mm Hg by regulating the percentage of isoflurane for a period of 60 minutes. The filament was then withdrawn to allow reperfusion and discontinuation of the isoflurane restored blood pressure. All wounds were closed, and the animals were allowed to recover in a room maintained at 21.6°C to 21.8°C. Sham-operated animals underwent the same surgical treatment without suture insertion or MABP reduction. In some experiments, a separate sham hypotensive group was also studied. This sham hypotensive group was identical to the normotensive one except for MABP, which was reduced to 60 mm Hg for 60 minutes after isolation of the internal carotid. All other physiological parameters were similar in MCAO, sham normotensive, and sham hypotensive rats, both before and immediately after surgery. Manipulation of the carotid body was identical in all sham and ischemic groups. When follow-up periods exceeded 24 hours, a subcutaneous bolus of 10 mL 0.9% NaCl was given immediately after surgery and daily for 2 days to prevent dehydration. Neurological assessment was performed in all rats before surgery and at the moment of euthanization, using clinical scales described previously. There was a 30% to 40% decrease in sensory and motor activity in rats undergoing MCAO. No neurological abnormalities were observed in sham and sham hypotensive groups.

Experiment 1: Blood Gases, Plasma Epo, and Hematological Changes After Reperfusion

A total of 4 to 5 animals in left sham-operated groups and 5 to 6 animals in left MCAO groups were killed at 8 different time intervals.
(0, 1, 2, 4, 6, 18, and 24 hours) after reperfusion. The animals were
anesthetized and blood from the tail artery was used for measurement
of blood gases, followed by cardiac puncture for measurement of
Epo levels and complete blood count (CBC). Plasma Epo levels were
measured by competitive radioimmunoassay using the Epo-Trac 125I
RIA kit (DiaSorin).20 CBC was performed with a counter analyzer
(Coulter), and white blood cell (WBC) differential count was
measured by light microscopy on peripheral blood smears stained
with Wright–Giemsa.

Experiment 2: Hematological Changes 0 to 28
Days After Reperfusion
MCAO was induced in the left or the right middle cerebral artery.
Changes in hematological parameters were measured at 0, 2, 7, 14,
and 28 days after reperfusion. A total of 10 to 15 animals in each of
the left or right sham-operated groups and 5 to 9 animals in each of
the left or right MCAO groups were killed at each time point. Blood
was harvested by intracardiac puncture, and CBC and WBC differ-
ential count were performed as described above.

Statistical Analysis
After data validation and normality checks, the effects of the main
factors were assessed by means of a mixed ANOVA model with a
first-order autoregressive covariance structure. Main treatment ef-
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Results
Effect of MCAO on Blood Gases
After Reperfusion
There was a precipitous drop in SaO2 immediately after
surgery in both MCAO and sham groups because the animals were no longer oxygenated and ventilated (Figure
1). SaO2 stabilized at ~85% in sham animals 6 hours after reperfusion but continued to drop to 75% to 80% in MCAO
animals, with a significant difference between these groups at
24 hours (P=0.03). The average SaO2 values for the period 0
to 4 hours after reperfusion were virtually identical in the 2
groups (Figure 2A; 94.3±1.2% versus 93.7±1.8% in sham
and MCAO rats, respectively). Between 6 and 24 hours after
reperfusion, average SaO2 values were significantly lower in
MCAO rats and were in the hypoxic range (86±0.8% versus
78.8±1.7%; P=0.0005; Figure 2A). PaO2 changes were
similar to those of SaO2, with no difference between MCAO
and sham animals 0 to 4 hours after reperfusion and signifi-
cantly lower PaO2 levels in MCAO rats between 6 and 24
hours after reperfusion (66.7±1.4 mm Hg versus
58.0±2.0 mm Hg; P=0.0013; Figure 2B). There was no
significant difference in PaCO2 values between sham and
MCAO animals, although there was a trend for PaCO2 from
6 to 24 hours after reperfusion to be higher in the MCAO
group (P=0.0551; Figure 2C).

| Table 1. Time Course of Hematological Changes During the First 24 Hours After Reperfusion in MCAO and Sham Rats |
|---|---|---|---|---|
| Reperfusion Time (Hours) | Group | RBC (10^12/L) | Hematocrit (L/L) | WBC (10^9/L) |
| 0 | Sham | 5 | 6.41±0.28 | 0.38±0.02 | 8.80±1.01 |
| | MCAO | 5 | 5.94±0.52 | 0.34±0.03 | 8.98±2.14 |
| 1 | Sham | 5 | 5.53±0.21 | 0.32±0.01 | 6.42±0.66 |
| | MCAO | 5 | 5.81±0.19 | 0.33±0.01 | 6.32±0.73 |
| 2 | Sham | 4 | 6.31±0.13 | 0.36±0.00 | 9.50±0.80 |
| | MCAO | 5 | 6.06±0.20 | 0.35±0.01 | 7.84±0.83 |
| 4 | Sham | 5 | 6.21±0.11 | 0.35±0.01 | 12.50±2.04 |
| | MCAO | 5 | 6.58±0.33 | 0.37±0.02 | 9.12±0.84 |
| 6 | Sham | 4 | 5.51±0.51 | 0.32±0.03 | 10.80±1.26 |
| | MCAO | 5 | 6.48±0.46 | 0.36±0.03 | 9.72±0.62 |
| 18 | Sham | 5 | 6.76±0.15 | 0.39±0.01 | 10.90±1.20 |
| | MCAO | 5 | 6.74±0.36 | 0.39±0.02 | 9.64±1.15 |
| 24 | Sham | 5 | 6.88±0.23 | 0.39±0.01 | 9.10±0.89 |
| | MCAO | 6 | 7.17±0.21 | 0.40±0.01 | 6.74±0.72* |

Values are expressed as mean±SEM.
*Different from sham group (P<0.05, ANOVA followed by Wilcoxon rank–sum test).
Effect of MCAO on Peripheral Epo Production and Hematological Parameters After Reperfusion

Epo concentrations were similar in both groups at time 0, and they increased gradually with time in both cases, followed by a decline toward baseline levels. Peak concentration of Epo was 20% higher in MCAO animals (89.2 ± 8.9 mU/mL versus 74.4 ± 3.1 mU/mL; P < 0.05; Figure 3) and occurred 2 hours later (6 versus 4 hours after reperfusion). As well, plasma Epo levels in MCAO rats tended to have a negative correlation with SaO2 values in these animals (r = −0.62; P = 0.056). Changes in hematological parameters during the first 24 hours after reperfusion are summarized in Table 1, with no difference between groups except a 26% decrease in WBC count at 24 hours in MCAO rats compared with sham (P < 0.05).

Discussion

This study shows that MCAO rats develop moderate hypoxia (SaO2 ≤ 80%; PaO2 ≤ 60%) from 6 to 24 hours after reperfusion. The SaO2 and PaO2 decreases in ischemic animals are significant not only compared with sham controls but because these values are now at levels that compromise oxygen delivery.21 The poststroke systemic hypoxia in MCAO rats correlates well with previous clinical observations in stroke patients.1,3 Other studies using a transient MCAO model in Wistar rats have found no difference in PaO2 up to 4 hours after reperfusion,22 but no measurements were made after the 4-hour time frame.

In the ischemic brain, where autoregulation is often impaired, even low or moderate hypoxia can produce detrimental effects.23,24 The causes of poststroke hypoxia may be multiple, including alterations in the central regulation of respiration, weakness of the respiratory muscles on the
hemiplegic side, and obstructive sleep apnea. Medical complications arising after stroke such as aspiration, chest infection, and pulmonary embolism may also impair oxygenation. In our model, hypoxia is not likely caused by aspiration pneumonia or pulmonary embolism because the animals were not infected or immobile. The low PaO2 associated with slightly increased PaCO2 points to a problem of ventilation, such as respiratory muscle weakness, central respiratory dysfunction, or sleep apnea. It is also possible that MCAO animals had a greater respiratory depressant response to anesthesia because of progressive underlying brain damage. The immediate response to hypoxia involves reflex hyperventilation mediated by chemoreceptors in the carotid body. Manipulation of the carotid body during surgery was identical in the sham and MCAO groups, thus eliminating chemoreceptor damage as a possible reason for the difference in PaO2 between the 2 groups.

Hypoxia is the main stimulus of Epo production in both peripheral and brain tissues by activation of the transcription factor hypoxia-inducible factor-1α. A prolongation of the increase over time of plasma Epo is observed in MCAO rats 6 hours after reperfusion, followed by moderate polycythemia 2 to 7 days after reperfusion. The fact that Epo levels increased only transiently in the presence of persistent hypoxemia is consistent with observations in other models and this may be explained, at least in part, by cellular adaptation to hypoxia. The early course of time of Epo increase is also consistent with the known effects of hypoxemia and Epo increase on erythropoiesis in rats. The maximal increases in plasma Epo, RBC count, and hematocrit in MCAO rats are 20% to 30% over sham controls. These responses remain below the magnitude required to increase blood viscosity or contribute to cerebral infarction. As well, the amounts of brain Epo induced by MCAO are also relatively low and likely insufficient to protect against acute cerebral ischemia. However, they may play a neuroprotective role in tolerance to further stroke episodes by mechanisms involving prevention of neuronal cell death through inhibition of apoptosis. By analogy, the moderate polycythemia observed in MCAO rats might represent an attempt to precondition the brain against subsequent ischemia.

Systemic hypotension is a feature of the MCAO model but not present in the sham group. The results of the sham hypotensive group eliminate hypotension as a contributor to the observed hematological changes, and previous studies have demonstrated that controlled mild hypotension does not affect systemic oxygen delivery to tissues. Therefore, hypoxia and the changes in hematological parameters in the stroke group appear independent of hypotension.

It is possible that the increase in hematocrit is simply the result of dehydration and homoconcentration, but this is unlikely. Hydration was assured by subcutaneous administration of saline, and if hematocrit increased as a result of dehydration, a concomitant increase in WBC count would be seen as well. In this study, the increase in RBCs and hematocrit was accompanied by a concomitant decrease in WBC, which is inconsistent with dehydration. Future studies measuring reticulocyte counts will provide additional proof of true erythropoiesis. Interestingly, a decrease in spleen white cells was observed previously in this same model, indicating that white cells may shift from both blood and the spleen into other lymphoid or nonlymphoid tissues, including the ischemic brain. Alternatively, increased cell death may play a role. To our knowledge, this is the first study relating systemic hypoxia to plasma Epo increase and erythropoiesis after MCAO. This supports the hypothesis that hypoxia occurs after cerebral ischemia and may affect outcome.

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