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Failure of normobaric oxygen therapy to reduce ischemic brain damage in rats

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Li Y, Kawamura S, Shirasawa M, Yasui N, Fukasawa H. Failure of normobaric oxygen therapy to reduce ischemic brain damage in rats. Undersea Hyperbaric Med 1994; 21(3):245–249.—We investigated the effects of 24-h oxygen therapy on focal cerebral ischemia in rats. Under halothane anesthesia, a 3-0 nylon thread was introduced into the neck internal carotid artery to occlude the left middle cerebral artery (MCA). Under atmospheric pressure, group 1 animals inhaled standard air; group 2, 40% O_2; group 3, 60% O_2; and group 4, 100% O_2. Neuropathologic outcomes were quantified after a 24-h inhalation period. Infarct volumes tended to decrease in groups 2–4, but the decreases were not significant when compared with group 1. Hemispheric volume differences of groups 2–4 (27 ± 18 mm^3, 22 ± 17 mm^3, and 31 ± 22 mm^3, respectively) were less than that of group 1 (61 ± 23 mm^3, $P < 0.05$). Our results demonstrate that O_2 therapy reduces brain swelling in rats 24 h after MCA occlusion. However, a dose-dependent decrease in brain swelling was not observed. In addition, we failed to see a significant decrease in the infarct size.

brain swelling, cerebral ischemia, middle cerebral artery occlusion, neuropathology, oxygen therapy, rats.

It is not clear whether inhalation of normobaric oxygen (O_2) is effective in the treatment of cerebral ischemia, because hyperoxemia can induce vasoconstriction and decrease cerebral blood flow (CBF) (1, 2). Because O_2 is used in stroke patients for respiratory complications, it is important to know whether there is also an effect of this form of normobaric O_2 application on the cerebral ischemia. O_2 is often inhaled for several days, although there is no quantitative neuropathologic information about the effects of O_2 therapy on focal cerebral ischemia when the therapy is performed for $\geq$24 h. This study was therefore conducted to investigate the effects of 24-h O_2 therapy on neuropathologic outcomes after occlusion of the middle cerebral artery (MCA) in rats.

MATERIALS AND METHODS

General preparation

We used 43 adult male, Sprague-Dawley rats (Charles River Japan, Inc.) weighing 250–290 g, having free access to food and water, and respiring spontaneously during

all procedures. Anesthesia was induced with 4% halothane, which was switched to 1.5% during the skin incision; it was then maintained with 1% halothane. The animals were allowed to inhale air or O_2 or both, as mentioned later. Rectal temperature was kept at 37°C with a heating pad. The tail artery was cannulated for continuous blood-pressure monitoring and for blood sampling. A ventilated desiccator (type-CR, Iuchi, Osaka, Japan) was used as a treatment box ($28 \times 29 \times 30$ cm^3), and each treatment gas was introduced into it at a rate of 10 liter/min. We put soda lime (Wako Chemical Industries, Ltd., Osaka, Japan) into the box to reduce the CO_2 content to <1,000 ppm. The O_2 and CO_2 concentrations were measured using a gas analyzer (ABL330, Radiometer, Copenhagen, Denmark) and a CO_2 analyzer (type-ZFP5, Fuji Electric Co., Ltd., Tokyo, Japan), respectively. The temperature and humidity in the box during treatment were 31° ± 2°C and 40 ± 10%, respectively.

Animal groups studied

Animals were randomly assigned to one of four groups. Group 1 ($n = 9$) served as controls and inhaled air (21% O_2). Group 2 ($n = 11$) and group 3 ($n = 11$) inhaled an air/O_2 mixture (40 and 60% O_2, respectively). Group 4 animals ($n = 12$) inhaled 100% O_2. Two to three animals received the same treatment at the same time. The O_2 concentrations during anesthesia and treatment were completely controlled. The CO_2 concentration decreased according to the step-wise increases in the O_2 concentration, except for the outflow CO_2 concentration (from the treatment box), which was similar in all four groups. The CO_2 concentrations averaged 330–370 ppm in group 1, 284–296 ppm in group 2, 197–217 ppm in group 3, and a trace in group 4.

Operative technique

The left MCA was occluded as described previously (3). Through a ventral midline incision, the origin of the pterygopalatine artery was occluded temporarily with a microclip. The external carotid artery was ligated and cut, and a clip was placed temporarily at its origin. The stump of the external carotid artery was punctured with a needle, and a 3-0 nylon thread was introduced into it. A silk suture around both the arterial stump and the intra-luminal thread was tightened to prevent bleeding, and the clip on the stump was removed. The nylon thread was advanced into the internal carotid artery, and the MCA origin was occluded. The clip on the pterygopalatine artery was removed, and the skin incision was closed. The catheter in the tail artery was filled with heparin and heat-sealed. The animals were removed quickly to the treatment box. There was zero minute between the operative procedures and introduction into the treatment box. None of the animals had seizures and there was no mortality.

Neuropathology

Twenty-four hours after the MCA occlusion, animals were anesthetized with 1.5% halothane. Rectal temperature was maintained at 37°C by a heating pad, and the tail artery served for blood-pressure monitoring and blood sampling. After heparinization, transcardiac perfusion fixation was performed with 10% buffered formalin, and the brain was stored in 15% buffered formalin.

From the forebrain embedded in paraffin wax, coronal sections (5-μm thick) were obtained and stained with hematoxylin-eosin and examined by light microscopy. Area measurements were done at eight coronal levels, and the levels were chosen at 1.5-mm intervals. The first level was 2.5 mm posterior to the frontal tip. Areas of infarction were measured using an image analyzer (Texture Analyzing System, Leitz, Wetzlar, Germany). Measurements were performed separately for the pallium, the striatum, and each hemisphere. The volume of infarction was calculated with a computer program by summing the infarcted areas of sequential sections, and by multiplying that sum with the total interval thickness between sections. The volume of each hemisphere between the eight levels was also calculated. The amount of infarct was expressed both as a percent of the total cerebral volume (% infarct volume) and as an absolute term (mm^3). Because the measurements of infarct volume included the effect of brain swelling, we estimated the hemispheric volume difference (mm^3) by subtracting the volume of the right (non-ischemic) hemisphere from that of the left (ischemic) hemisphere (4). Our previous study has shown that the hemispheric volume difference in sham-operated animals ($n = 5$) was near zero (left per right hemispheric volume ratio = 101 ± 1%) (5).

Statistical analysis

All data were expressed as mean ± SD. A two-way analysis of variance and then the Scheffe's multiple comparison test were used to define statistically significant differences among the groups. A P value <0.05 was considered significant.

RESULTS

Physiologic variables during MCA occlusion and before death

The Pa_{O_2} increased step-wise according to the increase of the O_2 concentration in the inhaled gases: the Pa_{O_2} averaged 75–76 mmHg in group 1, 156–179 mmHg in group 2, 253–285 mmHg in group 3, and 483–523 mmHg in group 4. The other values were similar among groups: mean arterial blood pressure averaged 76–85 mmHg, Pa_{CO_2} averaged 43.9–50.9 mmHg, arterial blood pH averaged 7.36–7.43, and hematocrit averaged 41–43%, in all four groups.

Neuropathology

A linear regression analysis of the total infarct volume and the hemispheric volume difference was performed to study the overall relation between them. A significant linear correlation was found, as shown in Fig. 1. The hemispheric volume differences in groups 2–4 (27 ± 18 mm^3, 22 ± 17 mm^3, and 31 ± 22 mm^3, respectively) were smaller than that of group 1 (61 ± 23 mm^3, $P < 0.05$). The infarct volumes (% infarct volume) also tended to decrease in the O_2-treated groups (31.2 ± 8.3% in group 2, 28.2 ± 10.2% in group 3, and 31.2 ± 10.7% in group 4), although they were not

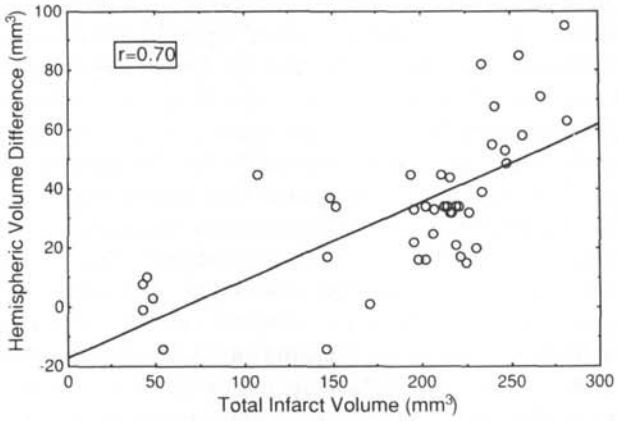

FIG. 1—Overall relation between the total infarct volume and the hemispheric volume difference.

different from that in group 1 (34.6 ± 8.5%). Neuropathologic findings among groups 2–4 were essentially the same.

DISCUSSION

Effects of normobaric oxygen on cerebral ischemia

This study demonstrates that O_2 therapy decreased the hemispheric volume difference (brain swelling) after MCA occlusion in rats that were treated with 40–100% O_2 for 24 h. However, O_2 therapy failed to show a dose-dependent protective effect against brain swelling. The infarct size also tended to decrease, although the decrease was not significant (compared with the air-treated control). Therefore, this study suggests that O_2 therapy under atmospheric pressure may not protect against ischemic brain damage in rats. The CO_2 concentrations in the inhaled gases (FI_{CO_2}) decreased in the O_2-treated groups, and the FI_{CO_2} changes may possibly affect CBF. However, the CO_2 tensions in the arterial blood (Pa_{CO_2}) were similar in all groups. Therefore, the lower FI_{CO_2} used in the present study may have a negligible effect on CBF.

The following discrepancy exists: The decrease of the infarct size was not significant, but the decrease in swelling was; this, in spite of there being a linear relationship. A tendency to decrease infarct size and hyperoxemia-induced vasoconstriction may explain this discrepancy.

In this study we used the hemispheric volume difference as an indicator of brain swelling. A similar value was used previously as edema volume by Brint et al. (4). They estimated brain swelling primarily due to edema (increase in brain water content) by subtracting the neocortical volume in the non-ischemic hemisphere from that in the ischemic hemisphere. Although the accumulation of edema fluid presumably plays a major role in brain swelling during the acute phase of ischemia, vasodilation also probably plays an important role. Infarct size is linearly correlated to brain swelling due to edema (6). However, we observed a discrepancy (mentioned above) between the infarct size and brain swelling. From our results, we believe that the observed decrease in brain swelling was due to both a tendency to decrease infarct size and hyperoxemia-induced vasoconstriction.

Time course of infarction and oxygen therapy

Recent studies have indicated that rat MCA occlusion with a duration of 3 h is sufficient to attain maximal infarction observed after permanent ischemia (6–8). The O_2 therapy may have a therapeutic effect on cerebral ischemia for only a short duration; for example, within 4 h after an ischemic insult (9). However, it is unlikely that O_2 therapy can decrease the infarct size after a longer period than 4 h, even if the therapy can delay infarction processes. This is the probable reason why the infarct volume assessed 24 h after the MCA occlusion did not show a significant decrease in the O_2-treated groups.

CONCLUSION

Our study demonstrates that 24-h O_2 therapy under atmospheric pressure reduces brain swelling in rats after MCA occlusion. However, a dose-dependent decrease in brain swelling was not observed. In addition, O_2 therapy does not reduce the infarct size significantly 24 h after the stroke.

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