Hyperbaric oxygen therapy in experimentally induced acute cerebral ischemia

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Shiokawa O, Fujishima M, Yanai T, Ibayashi S, Ueda K, Yagi H. Hyperbaric oxygen therapy in experimentally induced acute cerebral ischemia. Undersea Biomed Res 1986; 13(3):337–344.—Effects of hyperbaric oxygen (HBO) on acute cerebral ischemia were studied in spontaneously hypertensive rats, which had the carotid artery bilaterally ligated. The animals were exposed to HBO (100% O\textsubscript{2} at 2 ATA) for 30 min at 1 or 3 h after carotid ligation (treated group). Survival time and brain tissue metabolites were measured after HBO in these animals and compared with ischemic animals without HBO exposure (nontreated group). The animals treated at 3 h after ligation survived longer (6.5 ± 0.7 h) than did nontreated ones (4.3 ± 0.2 h) (P < 0.05). The cerebral lactate increased much less in these treated animals (24.60 ± 1.67 mM/kg) than in nontreated ones (31.78 ± 1.68 mM/kg) (P < 0.05). Cerebral ATP levels tended to decrease less in the former (0.66 ± 0.17 mM/kg) than in the latter (0.59 ± 0.07 mM/kg). When HBO started at 1 h after carotid ligation, however, there were no significant differences of survival time or brain metabolites between treated and nontreated groups of animals. The present results indicate that HBO administered at 3 h after brain ischemia prevents further increase in cerebral lactate and produces a slight but significant increase in survival time.

hyperbaric oxygen
spontaneously hypertensive rat
cerebral ischemia

Hyperbaric oxygen (HBO) has been used as one of the treatments for ischemic cerebrovascular disorders and traumatic brain edema. The use of HBO produces more dissolved oxygen in plasma, resulting in improvement of hypoxia in the ischemic tissue. It is also known that progression of brain edema can be suppressed by cerebral vasoconstriction due to high Po\textsubscript{2} in combination with the increased availability of oxygen (1, 2). Although many observations support the clinical (3–7) and experimental (8–14) usefulness of HBO, inappropriate use of HBO has adverse effects on the lung and CNS in experimental animals (15–19).

It is well known that hypertension is one of the major risk factors of cerebrovascular disease. Spontaneously hypertensive rats (SHR) (20) which we used in the present
study are generally considered to be a convenient model for hypertensive vascular disease, and we previously reported that bilateral carotid artery ligation produces severe supratentorial ischemia (21–23).

The present study was undertaken to clarify whether HBO has a beneficial effect on acute cerebral ischemia. For this purpose, survival time and brain tissue metabolites were determined in SHRs which were exposed to HBO at 2 time intervals following bilateral carotid occlusion.

MATERIALS AND METHODS

Survival protocol

Thirty-four male SHRs, weighing between 260 and 450 g, were anesthetized with ether. Both common carotid arteries were exposed through ventral midline incision, separated from the vagosympathetic trunks, and permanently ligated with silk sutures at the same time. After the skin incision was closed, the animals were returned to their cages.

The animals were randomly divided into 4 groups, 2 for HBO treatment and 2 for controls. Animals in the treatment groups were placed in a hyperbaric chamber at an oxygen pressure of 2 atmospheres absolute (ATA) for 30 min at either 1 h (1 h ischemia) or 3 h (3 h ischemia) after carotid ligation. Animals in the nontreatment group were always kept in the same condition as the treatment groups except for exposure to HBO. Survival time was defined as the time interval from carotid ligation to death. Since all the animals in the treatment groups survived at least 1.5 or 3.5 h after bilateral carotid ligation (BCL), animals in the nontreatment groups which died within 1.5 h (group of 1 h ischemia) or 3.5 h (group of 3 h ischemia) after BCL were excluded from data analysis.

Cerebral metabolites

Twenty-six male SHRs were used for the metabolic study. Surgical procedures, experimental protocol, and animal groups were substantially the same as those for the previous study. In this experiment, however, animals were reanesthetized with intraperitoneal amobarbital (50 mg/kg body weight) after the termination of 30 min HBO therapy. Then one femoral artery was cannulated for blood pressure recording and sampling for acid-base balance. A plastic funnel was fitted into a skin incision over the skull and the head was frozen in liquid nitrogen in situ 1 h after the completion of HBO therapy. Therefore, the time intervals from carotid ligation to brain freezing were 2.5 h for the 1 h ischemia group and 4.5 h for the 3 h ischemia group. The whole brain was chiseled out in a frozen state and separated grossly into supra- and infratentorial portions. In rapid sequence, the frozen brain was weighed and ground. After the addition of cold perchloric acid, the tissue homogenate was maintained at 0–4°C, centrifuged, and neutralized with potassium hydroxide at a pH between 4.5 and 5.0. Lactate and ATP concentrations in the tissue homogenates were determined by the standard enzymatic methods. Shortly before freezing the head, an arterial sample was obtained anaerobically for determinations of PCO₂, PO₂, and pH by an Instrumentation Lab (IL) model 113 meter.
Statistical analysis for data was performed using unpaired Student’s t test. All P values less than 5% were considered to be significant.

RESULTS

Survival

Figure 1 shows survival times in animals treated with HBO and the controls. The animals exposed to HBO 1 h after carotid ligation survived for 5.0 ± 0.5 (SEM) h and without HBO for 4.1 ± 0.3 h (P > 0.05). On the other hand, survival time in those exposed to HBO 3 h after ligation was 6.5 ± 0.7 h, which is longer than the 4.3 ± 0.2 h in nontreated ones (P < 0.05). Among HBO-treated animals, the average survival time was 1.5 h longer in the late-treated group than the early treated one, although the difference did not reach statistical significance.

Cerebral metabolites

Mean values for lactate and ATP concentrations in supratentorial and infarcted tectal portions of the brain are shown in Table 1. Compared with the nonischemic control value of our laboratory (1.82 ± 0.10 mM/kg) (24), supratentorial lactate levels were extremely high in both nontreated and HBO-treated groups (24.36 ± 3.40 mM/kg) after 2.5 h ischemia. With 4.5 h of ischemia, lactate increased further to 31.78 ± 1.68 mM/kg in the nontreated group, but stayed at near the same level of 24.60 ± 1.67 mM/kg in the

![Survival Time Graph]

Fig. 1. Survival time following bilateral carotid ligation in SHR treated with or without HBO at 1 or 3 h ischemia.
TABLE 1
SUPRA- AND INFRATENTORIAL METABOLITES OF THE ISCHEMIC BRAINS
WITH OR WITHOUT 30 MIN OF HBO TREATMENT STARTED AT 1 OR 3 h AFTER
CAROTID LIGATION

<table>
<thead>
<tr>
<th>Duration of Ischemia</th>
<th>2.5 h</th>
<th>4.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBO Treatment No. of Rats</td>
<td>None 7</td>
<td>at 1 h 6</td>
</tr>
<tr>
<td>Supratentorium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (mM/kg)</td>
<td>25.10 ± 2.29</td>
<td>24.36 ± 3.40</td>
</tr>
<tr>
<td>ATP (mM/kg)</td>
<td>0.73 ± 0.12</td>
<td>0.86 ± 0.19</td>
</tr>
<tr>
<td>Infratentorium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (mM/kg)</td>
<td>4.85 ± 1.02</td>
<td>4.17 ± 0.44</td>
</tr>
<tr>
<td>ATP (mM/kg)</td>
<td>1.59 ± 0.36</td>
<td>1.79 ± 0.18</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; statistical significance indicated by *(P < 0.05) (vs. nontreated of 2.5 h ischemia), **(P < 0.05) (vs. nontreated of 4.5 h ischemia).

Lactate (mM/Kg)

Fig. 2. Supratentorial lactate concentrations of ischemic brain in SHR treated with or without HBO at 1 or 3 h after carotid ligation.

Treated one (P < 0.05 vs. nontreated). Figure 2 shows the individual lactate values in each group. On the other hand, supratentorial ATP decreased markedly in 2.5 h ischemia and to a further extent in 4.5 h ischemia. ATP reductions were slightly smaller in HBO-treated groups than corresponding nontreated ones, but their differences did not reach statistical significance.
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Infratentorial metabolites changed similarly but less markedly, as did supratentorial metabolites, namely, lactate increased and ATP decreased after carotid ligation. There was also a tendency for a smaller increase in lactate and decrease in ATP in HBO-treated groups, compared with nontreated ones.

Table 2 summarizes the average values for mean arterial pressure (MAP) and arterial acid-base balance. The MAP tended to be lower in nontreated groups than treated ones and similarly in 4.5 h ischemia than in 2.5 h ischemia. Arterial \( \text{Po}_2 \) and \( \text{PCO}_2 \) were slightly different among the groups but such differences were neither consistent nor significant. All 4 mean pH values are virtually identical.

DISCUSSION

The animal model of cerebral ischemia used in this study (21, 22, 25, 26) has the following characteristics. Cerebral blood flow is reduced to less than 17% of control following BCL in SHR (27), but the cerebral ischemia induced is not complete. Such an ischemia is mainly located in the supratentorium in the early stages and extended to the infratentorium later (23). Ischemic changes of the brain in these animals are reversible by recirculation of the occluded carotid arteries at 3 h or less after BCL. This was previously proven metabolically and histologically. Since supratentorial lactate and ATP concentrations of normal brain in SHR averaged 1.82 and 2.2 mM/kg, respectively (24), the present metabolic data demonstrating an excessive increase in lactate and a marked decrease in ATP with time indicate full induction of brain ischemia by BCL in this study.

The use of HBO therapy 3 h after BCL led to a slight but significant prolongation of survival time compared with that in nontreated ischemic animals, suggesting that HBO may be beneficial in the treatment of acute cerebral ischemia. Although the efficacy of HBO on brain damage has been explained by such reasons as oxygenation of ischemic tissue, suppression of brain edema, and reduction of red cell agglutination (28), a precise mechanism is still uncertain. An examination of brain tissue metabolites and lactate concentrations in both supra- and infratentorium showed an increase with time following carotid ligation, but the increase was significantly suppressed by HBO.

<table>
<thead>
<tr>
<th>Duration of Ischemia</th>
<th>HBO Treatment No. of Rats</th>
<th>2.5 h</th>
<th>4.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBO Treatment</td>
<td>None</td>
<td>7</td>
<td>6</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th></th>
<th>2.5 h</th>
<th>4.5 h</th>
<th>2.5 h</th>
<th>4.5 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mmHg)</td>
<td>159 ± 9</td>
<td>175 ± 18</td>
<td>131 ± 6</td>
<td>142 ± 13</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.02</td>
<td>7.44 ± 0.03</td>
<td>7.45 ± 0.02</td>
<td>7.43 ± 0.03</td>
</tr>
<tr>
<td>( \text{PCO}_2 ) (mmHg)</td>
<td>25.8 ± 2.1</td>
<td>26.5 ± 1.1</td>
<td>22.9 ± 1.0</td>
<td>26.6 ± 1.1</td>
</tr>
<tr>
<td>( \text{PO}_2 ) (mmHg)</td>
<td>97.1 ± 8.3</td>
<td>93.4 ± 2.1</td>
<td>106.8 ± 3.7</td>
<td>96.8 ± 4.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.
treatment 3 h after ischemia. In addition, ATP in the treated group tended to be higher than that in the nontreated one, although its difference did not reach a statistical significance, indicating that an anaerobic glycolysis is improved by HBO but a full recovery of the aerobic metabolism is not achieved. The present metabolic data suggest that prolonged survival in these treated animals is due to suppression of a further increase in lactate rather than to restoration of reduced ATP. Considering that acid metabolites such as accumulated lactate in the brain tissue after brain ischemic insult lead to a further deterioration of brain circulation as well as metabolism, it can be seen that suppression of increased lactate by HBO causes some increase in survival time.

When HBO was started at 1 h after BCL, however, there was little therapeutic effect on ischemic brain damage based on the brain metabolism and survival time. The reason is not certain, but it is possible that supratentorial lactate might increase rapidly during the 1 h period from discontinuation of HBO to freezing of the brain. In nontreated animals, lactate increased to approximately 14 times that of normal brain during the first 2.5 h of induced ischemia, whereas it increased only 17 times during the entire 4.5 h of ischemia, indicating that ischemic metabolites increase more in the early than the later stage of induced ischemia. This may be partly explained by the decreased supply of glucose, which is a major substrate of lactate in an anaerobic condition, and to ischemic tissue due to reduced residual cerebral blood flow with time. Cerebral lactate would supposedly decrease during and immediately after HBO treatment at 1 h, but it might increase very rapidly and progressively after discontinued HBO compared with the HBO group 3 h after BCL, and finally reach almost the same level in nontreated animals.

The effects of HBO have been reported to vary by pressure, duration of pressurization, humidity (29), and temperature (30) in the chamber. However, it is more important whether tissue damage before HBO is reversible and whether the cell recovered from ischemic injury is able to utilize oxygen normally. When oxygen is supplied again to tissue that has lost the ability to use it, a strong oxidative effect acts adversely and the tissue metabolism deteriorates; this is known as free radical reaction. However, we did not find any such harmful effect of HBO in this study, probably because we applied HBO 3 h after ischemia. This is a critical time for metabolic and histologic reversibility from ischemic damages in our animal model (24). Holbach et al. (13) have described HBO at 1.5–2.0 ATA to be ideal from the standpoint of glucose metabolism. Presently, HBO at 2 ATA for 30 min appears to be effective, but more appropriate conditions of HBO should be investigated further.

Either HBO or recirculation of the occluded arteries may have similar effects on the ischemic brain from the point of view that both could increase the oxygen supply to the ischemic tissue. Recirculation, however, leads to a rise in the intracranial pressure (ICP), brain edema, or hemorrhagic infarction, whereas HBO therapy is unlikely to have such adverse effects. On the contrary, high Pao2, which causes vasoconstriction of the cerebral arteries might result in diminution of ICP, suppression of brain edema, and recovery of brain metabolism as was shown in this experiment. Therefore, HBO is thought to be useful for cerebral ischemia in some cases.

It is concluded from the present results that HBO therapy administered 3 h after brain ischemia may be a beneficial means for protecting against a further increase in cerebral lactate, resulting in a small but significant improvement of survival time.
HYPERBARIC OXYGENATION IN CEREBRAL ISCHEMIA

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Shiokawa O, Fujishima M, Yanai T, Ibayashi S, Ueda K, Yagi H. Thérapie à l’oxygène hyperbare dans l’ischémie cérébrale aigue induite expérimentalement. Undersea Biomed Res 1986; 13(3): 337–344.— Les effets de l’oxygène hyperbare (HBO) sur l’ischémie cérébrale aigue furent étudiés avec des rats naturellement hypertensifs chez lesquels l’artère carotide fut ligaturée bilatéralement. Les animaux furent soumis à l’HBO (100% O2 à 2 ATA) pendant 30 min, soit 1 h ou 3 h après la ligature des carotides (groupe traité). Le temps de survie et les métabolites des tissus cérébraux furent mesurés après le traitement à l’HBO chez ces animaux et comparés avec des animaux rendus ischémiques mais non exposés à l’HBO (groupe non traité). Les animaux traités 3 h après la ligature survécurent plus longtemps (6.5 ± 0.7 h) que ceux non traités (4.3 ± 0.2 h) (P < 0.05). Le lactate cérébral augmenta beaucoup moins chez ces animaux traités (24.60 ± 1.67 mM/kg) que chez ceux non traités (31.78 ± 1.68 mM/kg) (P < 0.05). Les niveaux d’ATP cérébraux eurent tendance à diminuer moins chez le premier groupe (0.66 ± 0.17 mM/kg) que dans le dernier (0.59 ± 0.07 mM/kg). Toutefois, lorsque le traitement à l’HBO commença 1 h après la ligature carotidienne, il n’y eut pas de différences significatives dans le temps de survie ou les métabolites cérébraux entre les groupes d’animaux traités et non traités. Les résultats présentent indiquent que l’administration de HBO 3 h après l’ischémie empêche l’augmentation supplémentaire du lactate cérébral et produit une augmentation légère mais significative du temps de survie.

oxygène hyperbare
rat naturellement hypertensif
ischémie cérébrale

REFERENCES