The effects of hyperbaric oxygen treatment on lipid peroxidation of pregnant rabbits and their fetus during late pregnancy.

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Submitted 9/7/2005; final copy accepted 1/24/2006

Objective: To measure the effects of hyperbaric oxygen on lipid peroxidation of pregnant and fetal rabbits during late pregnancy. Methods: Sixteen pregnant rabbits were randomly and equally divided into two groups. One, the HBO₂ group, was exposed to 2-atm oxygen for 60 min a day from the 21st to the 30th day of gestation, and the other group, non-HBO₂ group, did not obtain any hyperbaric oxygen treatment. Results: On the 30th day of pregnant period, the activity of antioxidant enzyme SOD in plasma of the pregnant rabbits of the HBO₂ group was significantly higher than that of the non-HBO₂ group, but there was no significant difference in the level of oxidative stress marker 8-iso-PG-F₂α between the two groups. As for the fetal rabbits, the SOD activity in umbilical plasma, placenta tissue and fetal brain tissue of HBO₂ group was significantly higher than that of the non-HBO₂ group, while there was no statistical difference between the concentrations of 8-iso-PG-F₂α of HBO₂ and non-HBO₂ group. Conclusion: HBO₂ treatment during the late pregnancy up-regulates the activity of antioxidant enzyme in plasma of pregnant rabbits, placenta, umbilical plasma, and fetal brain. This does not have significant effect on the oxidative stress in these tissues.

INTRODUCTION

Since the late 70s, some authors in the former Soviet Union have tried to treat both acute hypoxia in labor and fetal growth restriction secondary to placental insufficiency with hyperbaric oxygen (HBO₂). The research found that HBO₂ was invaluable in improving both placental blood flow and O₂ diffusion at the cellular level (1). As we previously reported, HBO₂ therapy could reduce the values of systolic/diastolic ratio and the pulse index in umbilical arteries of late-onset fetal growth restriction patients, could improve the uteroplacental microcirculation and neonatal birth weight. HBO₂ is an effective method for the treatment of late-onset fetal growth restriction (2). However, HBO₂ can also cause the formation of oxygen free radicals, and the risk of oxygen toxicity from HBO₂ treatment, especially during pregnancy is controversial. Besides, there are few articles describing the relationship between HBO₂ protocols and oxidative stress. So, we conducted the present experimental study to assess the status of oxidative stress in pregnant rabbits and their fetus treated with HBO₂ during late pregnancy.

MATERIALS AND METHODS

All experimental procedures were approved by the local animal care committee. Sixteen healthy timed-pregnant New Zealand white rabbits purchased from Guangdong Animal Research Center (Guangdong, China) were randomly and equally divided into two groups and were respectively given either
the normal room-air treatment (non-HBO\textsubscript{2} group) or the HBO\textsubscript{2} treatment (HBO\textsubscript{2} group), respectively. During the 21st to 30th day of gestation, the HBO\textsubscript{2}-group treated rabbits were placed daily into a medical multiplace chamber (NG280-900, Lingbo Zhejiang China) pressurized to 2 atm within 10 minutes where they remained for 60 minutes. Non-HBO\textsubscript{2} rabbits were also moved into the same chamber not to be pressurized, and they breathed room air.

On the 20th day of gestation, (i.e. one day before the rabbits were moved into the chamber) and the 30th day when the rabbits were leaving the HBO\textsubscript{2} chamber, 4mL of venous blood was drawn from pregnant rabbits in both groups. On the 30th day (at term), after the blood samples were taken, we performed cesarean section for the pregnant rabbits under pentobarbital sodium anesthesia. Two fetuses from each pregnant rabbit were randomly selected for the study. They were sacrificed by decapitation, and a blood sample was taken from umbilical cord venous. All blood samples were collected in heparin-coated test tubes containing 10μM of indomethacin to prevent in\textit{vitro} formation of 8-iso-PGF\textsubscript{2α}, and centrifuged for 10 min at 3000 g and 4 °C for 10 min. The supernatant was immediately used for the assays of total levels of 8-iso-PGF\textsubscript{2α} and SOD by the same method described above. All experimental samples were analyzed in duplicate.

Results presented are means ± SD. Data was analyzed using the SPSS version 10.0 statistical package (Chicago, IL). The unpaired or paired Student's t-test was used when two variables were compared, and P<0.05 was considered statistically significant.

**RESULTS**

**Plasma 8-iso-PGF\textsubscript{2α}/SOD levels in pregnant rabbits and their fetus of two groups.**

In the 30th-day pregnant rabbits and their fetus, there was no significant difference in plasma 8-iso-PGF\textsubscript{2α} levels between the HBO\textsubscript{2} and non-HBO\textsubscript{2} group (P>0.05). The total 8-iso-PGF\textsubscript{2α} concentrations of both non-HBO\textsubscript{2} and HBO\textsubscript{2} groups on the 30th day were significantly higher than those on the 20th day (P<0.01, Table 1). On the 30th day, the value of SOD in non-

<table>
<thead>
<tr>
<th>Groups</th>
<th>On 20th day pregnancy</th>
<th>On 30th day pregnancy</th>
<th>Fetal umbilical plasma</th>
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<tbody>
<tr>
<td>n</td>
<td>8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Non-HBO\textsubscript{2}</td>
<td>597.62±55.79\textsuperscript{a}</td>
<td>666.10±53.49</td>
<td>578.17±51.71\textsuperscript{a}</td>
</tr>
<tr>
<td>HBO\textsubscript{2}</td>
<td>584.41±65.64\textsuperscript{a}</td>
<td>648.58±50.32</td>
<td>561.16±58.01\textsuperscript{a}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}: P<0.01, compared to the value on 30th day pregnancy.
HBO₂ group was 273.72±18.44U/ml, which was lower than that of HBO₂ group (388.01±41.91 U/ml). The fetal umbilical plasma SOD level of the HBO₂ group was significantly higher than that of the non-HBO₂ group. In the non-HBO₂ group, the SOD activity of pregnant rabbits on the 30th day was higher as compared to that on the 20th day (Table 2).

### Table 2. The plasma SOD activity (U/ml) in pregnant rabbits and their fetus of two groups (X±SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>On 20th day pregnancy</th>
<th>On 30th day pregnancy</th>
<th>Fetal umbilical plasma</th>
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<tbody>
<tr>
<td></td>
<td>n 8</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Non-HBO₂</td>
<td>246.43±13.89*</td>
<td>273.72±18.44</td>
<td>257.29±14.62</td>
</tr>
<tr>
<td>HBO₂</td>
<td>248.03±15.87*</td>
<td>388.01±41.91</td>
<td>373.41±40.29*</td>
</tr>
</tbody>
</table>

*: P<0.01, compared to the value on 30th day pregnancy;  
*,: P<0.01, compared to non-HBO₂ group;

**DISCUSSION**

The effect of HBO₂ on lipid peroxidation of pregnant rabbits during the late pregnancy.

Oxidative stress occurs when the formation of oxygen free radicals exceeds the antioxidant defense capabilities. Assessment of oxidative stress in vivo usually relies on the determination of stable oxidative modified molecules. 8-iso-PGF₂α is a nonenzymatic and stable end product of lipid peroxidation, and several reports have indicated that 8-iso-PGF₂α is a reliable and sensitive marker of the free radical induced endogenous lipid peroxidation and oxidative stress in vivo(3).

HBO₂ treatment provides extra oxygenation of the tissues of the whole body, which may be associated with the production of oxygen free radicals and lipid peroxidation. Some authors (4,5) consider that HBO₂ therapy had beneficial effects on lipid peroxidation due to a decrease in the production of reactive oxygen species and increasing the antioxidant activity. However, Rocco M et al. (6) found that hyperbaric oxygenation in healthy volunteers induced lipid peroxidation, and TBARS levels showed a twofold increase in relation to the baseline during and after hyperbaric oxygenation. There are few reports about the effects of HBO₂ treatment on maternal oxidative stress during late pregnancy. Our findings showed that HBO₂ treatment was associated with a marked rise in maternal rabbit plasma SOD level during the late pregnancy, and the total plasma 8-iso-PGF₂α concentrations were not modified. This demonstrates that our HBO₂ protocol did not lead to increased maternal lipid peroxidation presumably due to its effect to enhance antioxidant activity.

Previous studies have shown that pregnancy is susceptible to oxidative stress, and that the increased lipid peroxidation (LPO) and reduced antioxidant activity may contribute to
the development of complications in pregnancy including preeclampsia, gestational diabetes and fetal growth restriction (7,8,9). The changes of lipid peroxidation and antioxidant enzymes throughout late pregnant period have not been fully described. Djordjevic A. et al. (9) showed that the concentration of lipid peroxidation marker thiobarbituric acid-reactive substance and activity of antioxidant enzyme SOD increased significantly in normal pregnant women as compared with non-pregnant women, which means that pregnant women are exposed to oxidative stress to a greater degree than non-pregnant women. In our experiment, the plasma SOD as well as 8-isoprostanes of pregnant rabbits on the 30th day was higher as compared to that on the 20th day. This indicates that lipid peroxidation of pregnant rabbits increased as gestational age advanced during the late pregnancy, although the activity of the main superoxide-scavenging enzyme increased in response to the elevated levels of oxidative stress during this period. The main reason for this change is uncertain. Recently, growing evidence suggests that placenta is the major source of pro-oxidant agents and antioxidant enzyme-systems (10). As the development of placenta, the production of lipid peroxidation increased gradually. Vanderlelie J. et al. (8) found a decreased enzymatic anti-oxidant capacity and increased oxidation in placental tissue from pre-eclamptic women. They considered that the oxidative stress of placenta might contribute to the pathogenesis of preeclampsia. Interestingly, in our experimental model, the SOD level in placental tissue increased in response to HBO\textsubscript{2} treatment, which suggested that HBO\textsubscript{2} therapy might become a useful tool to treat preeclampsia for its effect to enhance the enzymatic antioxidant capacity of placenta.

**The efficiency of HBO\textsubscript{2} therapy on fetal lipid peroxidation during late pregnancy.**

During the intrauterine period of life, fetal neuronal cells are more vulnerable to oxidative stress. Therefore, uncertainty arises in the possible adverse effect of HBO\textsubscript{2} treatment on the fetus. There are some case reports on short duration of HBO\textsubscript{2} treatment for carbon monoxide poisoning or air embolism occurrence in the women who also happen to be pregnant (11), most of whom show that the treatment with HBO\textsubscript{2} in their late pregnancy does not lead to adverse consequences (2). Our findings show that the administration of therapy dosage HBO\textsubscript{2} to pregnant rabbits during the late gestation leads to compensatory up-regulation of fetal plasma and brain antioxidant enzyme activities, without significant effect on the production of lipid peroxidation in fetus. As is known, fetus moves from an in utero hypoxic to a relatively hyperoxic environment, with an approximately 4-fold elevation in oxygen concentration, and the developmental changes in perinatal brain antioxidant enzymes are compensatory mechanisms to protect the newborn brain from oxidative stress (12). Thus we consider that HBO\textsubscript{2} treatment in mothers may benefit their offspring by enhancing the SOD activity of fetal brain.

In conclusion, our experiment indicated that the use of a therapeutic dose of HBO\textsubscript{2} during late pregnancy might be safe for the maternal-fetal lipid peroxidation and might be of medical significance as a method for the treatment of preeclampsia, gestational diabetes or fetal growth restriction. Further investigation is being continued in these areas.

**ACKNOWLEDGMENT**

This work was partially supported by grants No:[2002]254-7 from Department of Science and Technology of Guangdong Province and No:A2002332 from Grangdong Medical Research foundation.
REFERENCES