Platelet aggregation and release function in hyperbaric oxygenation

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Ersöz G, Oacakçioğlu B, Baştuğ M, Fıçicilăr H, Yavuzer S. Platelet aggregation and release function in hyperbaric oxygenation. Undersea Hyper Med 1998; 25(4):229–232.—The aim of the study was to investigate the acute and chronic effects of hyperoxynation on platelet aggregation and adenosine triphosphate (ATP) release. To observe the acute effects of hyperbaric oxygen (HBO₂), seven New Zealand rabbits were exposed to 2.4 atm abs oxygen for 90 min (group 1). Eight other rabbits were further exposed to O₂ daily for 20 days (group 2) to observe the chronic effects of HBO₂. Adenosine diphosphate (ADP) and collagen-induced platelet aggregation and ATP release were evaluated before and after oxygenation in groups 1 and 2. It was found that the maximal rate of ADP and collagen-induced platelet aggregation decreased after hyperbaric oxygenation in group 1. No significant alteration was observed in platelet responses at the end of 20 days of oxygenation in group 2. The adaptation of platelets to 2.4 atm abs O₂ after 20 days of exposure seems to need further investigation.

hyperbaric oxygenation, platelet aggregation, oxidative stress

It has been suggested that hyperbaric air and hyperbaric oxygen (HBO₂) affect blood coagulation (1). In previous studies that were performed in our laboratory, hypercoagulability was observed in New Zealand rabbits that were subjected to 5 atm abs of air (2). The cause of the hypercoagulability in relation to hyperbaric air and oxygen has been debated.

It is known that the generation of free O₂ derivatives increases under hyperoxic conditions (3,4). These derivatives play important roles in tissue injury (5), create a tendency to coagulation by endothelial damage, and affect the interaction between the endothelial layer and blood cells (6).

In vitro studies have shown that free oxygen radicals affect platelets directly (7,8). The effects of HBO₂ on platelet function have not yet been clarified. The aim of the study was to investigate the effects of acute and chronic application of 2.4 atm abs O₂ on platelet function.

MATERIALS AND METHODS

Animals and exposures: Fifteen New Zealand male rabbits (3–4 mo age, 1.6–2 kg weight) were used for the experiment. Two types of exposures were employed. Seven of the rabbits were subjected to 2.4 atm abs oxygen for 90 min (group 1). The remaining eight rabbits were exposed to 2.4 atm abs O₂ for 90 min daily for 20 days (group 2).

Aggregation and release measurements: Blood samples were obtained intracardially by using 19-gauge needles before and 1–2 min after the exposure at 0900–1100 h. They were put into propylene tubes containing 3.8% trisodium citrate (at a ratio of 1:9) and kept at room temperature for 20 min.

Platelet aggregation was evaluated by the electrical impedance method in whole blood by using the Chronolog model 560WB aggregometer (9). Two platinum electrodes were placed in the blood samples. Adenosine diphosphate (ADP) (10 μM) and collagen (2 μg/ml) were added to the samples for in vitro activation of platelets. Increase in impedance between the electrodes due to the adhesion and the aggregation of platelets on the electrodes was recorded. Maximal rate of platelet aggregation was determined by measuring the maximal slope of the aggregation curve. The adenosine triphosphate (ATP) release by platelets was measured by the luminescence channel of the aggregometer (9). Luciferin-luciferase was added to the samples, and the luminescence that radiated as a result of ATP and the luciferin reaction that was catalyzed by luciferase were measured. The luminescence channel was calibrated by 2 nM of ATP. The amounts of ADP and collagen-induced ATP release of platelets were determined by comparison with the curve obtained by 2 nM of ATP. ADP, collagen, ATP, and luciferin-luciferase reagents were obtained from Chronolog Corp (Havertown, PA).

Platelet counts were determined using the light microscope before and after exposure to the O₂. Platelet count, platelet aggregation, and platelet ATP release of the 15 rabbits were evaluated before exposure to O₂. The measurements were repeated in groups 1 and 2 after HBO₂.
Statistical analyses were performed using the Mann-Whitney U test.

RESULTS
The mean maximal rate of ADP and collagen-induced platelet aggregation, amount of ATP release and platelet count measured before HBO₂ and in groups 1 and 2 after HBO₂ are summarized in Table 1. The maximal rate of ADP and collagen-induced platelet aggregation decreased significantly \((P < 0.01, P < 0.05)\) after exposure to 2.4 atm abs O₂ for 90 min (Fig. 1). HBO₂ did not significantly alter either ADP and collagen-induced ATP release (Fig. 1) or platelet count. Ninety minutes of 2.4 atm abs oxygenation daily for 20 days did not affect ADP and collagen-induced platelet aggregation, ATP release, or platelet count (Fig. 2).

DISCUSSION
Hyperbaric oxygen therapy has been widely used for about 30 yr. Pulmonary and neurologic manifestations of O₂ poisoning are often cited as major side effects (10). It has been shown that daily exposure to O₂ at 2–2.4 atm abs for 1.5–2 h did not produce pulmonary symptoms (11,12).

The U.S. Air Force School of Aerospace Medicine reported that 20 daily HBO₂ therapies of 90 min O₂ breathing at 2.4 atm abs had no adverse effect and was accepted as the therapeutic dosage for O₂ (11). Therefore this protocol was chosen for the present study. Our animal subjects also did not show signs of toxicity during HBO₂.

Our results indicated that exposure to 2.4 atm abs O₂ for 90 min decreased ADP and collagen-induced platelet aggregation significantly. LaCroix et al. (1) found no change in any hemostatic variables measured when atmospheric pressure was increased from 1 to 3 atm abs. Similar results were obtained from the studies at 5.5 and 7 atm abs (1). Philip et al. (13) showed an increase in fibrinolytic activity, a decrease in plasminogen level, and a decrease in antithrombin III level in subjects who were exposed to 10.4 atm abs. It seems that hemostatic changes occur under extremely high atmospheric pressure. Partial oxygen pressure (PO₂) is approximately 2 atm abs at 10.4 atm abs of air. This PO₂ is near to the pressure that we used. Nevertheless, at these high atmospheric pressures, due to pulmonary squeeze, arterial PO₂ may not increase (14).

When O₂ is breathed at 2–3 atm abs pressure, the amount of O₂ dissolved in plasma increases 10–15 times (11).

Table 1: Mean Maximal Rate of Aggregation and Release of ATP From Platelets Induced by ADP and Collagen, and Platelet Counts Before and After Exposure to HBO₂ (*±SE)

<table>
<thead>
<tr>
<th></th>
<th>Before (group 1)</th>
<th>After (group 2)</th>
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</thead>
<tbody>
<tr>
<td>Max aggregation</td>
<td>ADP</td>
<td>12.03 ± 1.95</td>
</tr>
<tr>
<td>rate, ohm/min</td>
<td>collagen</td>
<td>11.72 ± 1.84</td>
</tr>
<tr>
<td>Release of ATP,</td>
<td>ADP</td>
<td>1.58 ± 0.34</td>
</tr>
<tr>
<td>nmol</td>
<td>collagen</td>
<td>2.09 ± 0.47</td>
</tr>
<tr>
<td>Platelet count</td>
<td>(×1,000/mm³)</td>
<td>400.4 ± 52.6</td>
</tr>
</tbody>
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*aSignificantly different from that measured before HBO₂ (\(P < 0.01\)). *bSignificantly different from that measured before HBO₂ (\(P < 0.05\)).

FIG. 1—(a) Maximal rate of ADP and collagen-induced platelet aggregation before and after 90 min, 2.4 atm abs oxygenation of group 1. (b) ADP and collagen-induced ATP release of the platelets before and after 90 min, 2.4 atm abs oxygenation of group 1.
PLATELET FUNCTION IN HYPERBARIC OXYGENATION

Although the production rates of free oxygen radicals increase in hyperoxic conditions (3,4), reports concerning the interaction between platelets and several oxidants have been conflicting (7,15). It is suggested that free radicals have dose-related effects on the platelets, and that hydrogen peroxide (H$_2$O$_2$) especially inhibits ADP-induced platelet aggregation at lower concentrations and increases the aggregation rate of platelets at higher concentrations (8). Ohyasaki et al. (7) reported that treatment of platelets with H$_2$O$_2$ (2–10 mM) caused decreases in the binding affinities of ADP and Ca$^{2+}$ and inhibited ADP-induced platelet aggregation. On the other hand, superoxide anions (O$_2^-$) enhance platelet adhesion and aggregation either through a direct effect on the platelets or indirectly by altering the formation of nitric oxide (NO) from endothelial cells (8).

Antioxidants are known to be regulators of platelet function (16). In our previous study it was also found that exposure to 2.4 atm abs O$_2$ for 90 min caused a significant decrease in the erythrocyte superoxide dismutase (SOD) activity in New Zealand rabbits. No alteration was observed in erythrocyte catalase (CAT) activity and plasma malondialdehyde (MDA) level. It was thought that exposure to O$_2$ at this level did not cause a severe oxidative stress (17).

Several conflicting results concerning the effect of HBO$_2$ on SOD activity have been noted. Ito et al. (18) suggested that levels of thiobarbiturate reactive substances, carbon-centered radicals, and SOD activity in the mitochondrial and cytosolic fraction of cerebrocortical homogenates of the rats increased after exposure to O$_2$ at 3 atm abs for 2 h. Harabin et al. (3) found that lung SOD activities of guinea pig and rat significantly increased after short intermittent exposure to O$_2$ at 2.8 atm abs for 4 h (10 min of O$_2$, 2.5 min of air). They reported that lung CAT and glutathione peroxidase (GPx) activities decreased after long exposures.

It was shown that CAT and GPx activities decreased after long exposures in the brain; no change in SOD activity was detected (3). The differences seem to be related to the different types of exposures and the tissues in which the antioxidants are measured (3). It was suggested that the decrease in SOD activity after HBO$_2$ might be due to the replications of cells poor in SOD, inhibition of the enzyme by its products, or decreased enzyme turnover (3). SOD activity is also related to the concentration of its substrate.

It was reported that HBO$_2$ caused NO generation and NO was an important mediator in oxygen toxicity (18,19). Ito et al. (18) reported that exposure to 3 atm abs HBO$_2$ for 2 h increased the level of arginine and it was thought that HBO$_2$ favors NO generation.

Nitric oxide reacts with O$_2^-$ to form biologically inactive nitrite and to produce peroxynitrite anion (ONOO$^-$) (8). Ma et al. (20) suggested that the rate of the NO–O$_2^-$ reaction was higher than that of the SOD–O$_2^-$ reaction. Finally it has been thought that a decrease in superoxide anion might occur due to the reaction with NO.

Both NO and its metabolite ONOO$^-$ that is produced as a result of the reaction with superoxide, stimulate the production of c-guanosine 5'-monophosphate (c-GMP) (19,21). It is known that c-GMP inhibits platelet activation (21).

In the present study, no alteration was observed in platelet functions in relation to chronic HBO$_2$ exposure. This result demonstrated that an adaptation to HBO$_2$ occurred within 20 days. The mechanism of adaptation of platelets to hyperoxygenation needs further investigation.

Hyperbaric oxygen therapy is generally applied to patients with pathologies associated with oxidative stress (22,23). It is known that these types of pathologies are usually accompanied by an inadequate antioxidant status. Thus the effects of HBO$_2$ have to be investigated in conditions with an inadequate antioxidant status.
The preliminary results of this study were presented at the XXth annual meeting of EUUBS in Istanbul in 1994.

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