Platelet function in humans is not altered by hyperbaric oxygen therapy.

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Thom SR, Fisher D, Stubbs J.M. Platelet function in humans is not altered by hyperbaric oxygen therapy. Undersea Hyperb Med 2006; 33(2):81-83. A pilot survey of platelet function was performed on 6 patients undergoing hyperbaric oxygen therapy (2.0 ATA O_2 for 2 hours, 6 days/week) for prophylaxis against osteoradionecrosis. Blood was drawn immediately prior to and after the first, tenth and twentieth treatment for measurements of platelet aggregation, ATP release and expression of activated αIIbβ3 integrin. No significant differences were observed due to hyperbaric oxygen exposures.

INTRODUCTION

This investigation was performed to determine whether a therapeutic hyperbaric oxygen (HBO_2) protocol perturbs platelet function in human beings. The study was prompted by a report documenting that in rabbits, a single exposure to HBO_2 (2.4 ATA O_2 for 90 minutes) will inhibit platelet aggregation and ATP release initiated by adenosine diphosphate (ADP) and collagen, but the effect does not re-occur after 20 daily treatments (1). The authors suggested that impaired platelet function may be a manifestation of oxidative stress, and the effect does not occur after repetitive treatments because HBO_2 induces antioxidant responses. HBO_2 has been shown to induce heat shock protein 70 and heme oxygenase-1 in circulating lymphocytes in humans (2,3). Other than the investigation by Ersoz *et al*. in rabbits (1), the only study on platelet function after HBO_2 was made by Seriakov and Feofanova (4). They found no effect on platelet aggregation among 65 patients undergoing 8 to 12 daily sessions of HBO_2 at 1.3 to 1.6 ATA for 40 minutes.

METHODS

Following IRB approval, we obtained blood from six patients undergoing prophylactic HBO_2 treatment for osteoradionecrosis. The protocol was daily exposure, six days per week, to 2.0 ATA O_2 for two hours with a 5 minute air break between each hour. These individuals were not using medications, had not consumed alcohol in the previous 24 hours, and had not taken aspirin or another non-steroidal anti-inflammatory agent in the previous 10 days. Three men and three women, mean age 53.5 $\pm$ 5.1 (SE) years old had 6 ml of venous blood drawn into polypropylene tubes containing 3.8% tri-sodium citrate (1:9 ratio) as anticoagulant. Studies on platelet function were performed at room temperature immediately after blood was obtained.

Platelet aggregation and ATP release were evaluated using a Chronolog model 560WB aggregometer (Chrono-log Corp., Havertown, PA). Blood (600 μl) was diluted with 300 μl phosphate-buffered saline (PBS) and 100 μl of 'Chrono-lume' solution (2 μM luciferin and 17,600 U/ml d-luciferase) purchased from the Chrono-log Corporation. Aggregation was assessed as an increase in impedance while adenosine triphosphate (ATP) release was measured in the luminescence channel of the instrument. Agonists used were 10 μM ADP, 5 μg/ml collagen and maximum ATP release was determined in the presence of 1 unit/ml thrombin. All reagents were purchased

from Chrono-log Corporation. Luminescence was calibrated using an aliquot of blood diluted as described above, and incubated with 2 nM ATP. Platelet counts were performed on blood samples using an automated counter (Coulter, Haileah, FL).

Surface expression of the activated form of αIIbβ3 integrin was determined by flow cytometry using an EPICS XL Flow Cytometer (Becton Dickinson, Franklin Lakes, NJ). Platelet samples were prepared by following procedures described by Shattil, *et al.* (5). In brief, within 5 minutes after blood was obtained, 5 µl blood was placed in polystyrene tubes containing 50 µl Hepes buffered saline (10 mM Hepes, 138 mM NaCl, 12 mM $NaHCO_3$, 10 mM KCl, 5.5 mM glucose, and 0.335 % bovine serum albumin, pH 7.4), plus a saturating concentration (20 µl) of fluorescein isothiocyanate (FITC) conjugated anti-PAC1 (BD Biosciences, San Diego, CA). Anti-PAC1 is an IgM antibody that only recognizes the activated form of the platelet αIIbβ3 integrin (CD41/CD61). Paired samples were incubated without or with 10 µM ADP to stimulate platelets and after 5 minute incubation at room temperature, samples were diluted to 500 µl with PBS and fixed with 1 % formalin. Platelets were identified in the flow cytometer by forward and side-light scatter profile. A gate was set around the platelets and PAC1 binding quantified on a logarithmic scale for 20,000 events.

RESULTS

Among the six test subjects, platelet counts were unaffected by HBO_2. Prior to the first treatment there were 1.6 ± 0.2 (SE) x 10^5 platelets/µl and 1.7 ± 0.2 x 10^5/µl after the two-hour treatment. Counts were not significantly different after the 10th and 20th treatments.

Maximum aggregation (ohms), the rate of aggregation (ohms/minute), and ATP release (nM) in response to collagen and ADP were not altered significantly by hyperoxia. Thrombin was used to assess maximum ATP release, but it could not be used to assess aggregation because it stimulates formation of a large fibrin clot. Results are shown before and after the first treatment in Table 1. There were no significant changes before or after the 10th and 20th treatments, and responses varied by less than 15 % over the course of therapy.

Activated αIIbβ3 integrin expression was assessed as the increase in fluorescence intensity when platelet suspensions were incubated in presence of 10 µM ADP. Prior to the first HBO_2 treatment there was an increase of 6.3 ± 0.8 (SE) fluorescence units and after treatment the increase was 7.7 ± 1.3 (no significant difference). Similar changes occurred after the 10th and 20th treatments.

DISCUSSION

We conclude that a course of twenty HBO_2 treatments at 2.0 ATA for 2 hours given six days/week has no significant effect on platelet function. While this is a null result, it is important to document whether typical protocols of HBO_2 modify physiological functions in humans. Admittedly, the sample size of the study was small, but because the variability in the data was also rather small, we could have detected relative differences between 25% and 50% for any of the platelet functions with a power of at least 0.8. It is notable that Ersoz, *et al.* (1) reported almost 80% impairment in aggregation rates and over 50% in some of the ATP release responses in their rabbit study. Absence of an effect in humans, versus the findings reported in rabbits, may be due to species differences or the fact that rabbit studies were conducted at 2.4 ATA O_2 (1). Saturation diving to a pressure of 2.8 ATA has been reported to inhibit platelet function (6). This could be an inert gas or hydrostatic pressure effect, or a stress response related to other environmental factors. LaCroix

et al. (7) have reported that a single exposure to 3 ATA O_2 does not alter circulating levels of hemostatic factors.

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