LIPID PEROXIDE-RELATED HEMODILUTION DURING REPETITIVE HYPERBARIC OXYGENATION

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INTRODUCTION

Tissue oxygen tension can be elevated under controlled conditions by simultaneously increasing the O₂ concentration of inspired air and the ambient pressure (Jamieson and Van Den Brank, 1963). Increasing tissue oxygen tension by hyperbaric oxygenation (HBO) reduces organ blood flow (Muhvich et al., 1992; Bergo and Tyssebotn, 1992) as well as some forms of edema (Nylander et al., 1985; Yamaguchi et al., 1990; Gehrs et al., 1991). An acutely reversible decrease of blood hemoglobin concentration by HBO (Bergo and Tyssebotn, 1992) further suggests hyperoxia affecting body fluid distribution.

Increasing tissue oxygen tension augments oxygen radical production (Freeman and Crapo, 1981; Freeman et al., 1982). HBO leads to oxidative damage by producing reactive O₂ intermediates (Jamieson et al., 1986; Thom and Marquis, 1987; Yusa et al., 1987; Zhang and Piantadosi, 1991). Lipid peroxidation seems to be an immediate cause of tissue damage (Hiramitsu et al., 1976; Torbati et al., 1991; Monstrey et al., 1991). Lipid peroxides also lead to vasoconstriction (Simon et al., 1990). HBO-induced vasoconstriction is prevented by antioxidants (Jacobson et al., 1992).

Brief episodes of lipid peroxidation can lead to persistent changes in cell function (Flohe et al., 1978; Kappus, 1985). Moreover, lipid peroxidation reaction products may diffuse away from the production site, leading to further damage at distant sites (Benedetti et al., 1979). Lipid peroxidation products and/or effects may, therefore, accumulate when, as under clinical conditions, HBO is repetitively applied. Renal blood flow remains depressed in rats.

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for at least 20 min after an acute HBO exposure (100% O₂, 202.6 kPa) of similar duration (Muhvich et al., 1992). Blood flow also remains reduced in some cerebral regions after HBO (Bergø and Tysebotn, 1992). Thus, it is not unlikely that repetitive HBO leads to sustained vasoconstriction implying corresponding changes in capillary filtration equilibrium. Red blood cell count (RBC), blood hemoglobin concentration (Hb), hematocrit (Hct) and plasma protein concentration (TPC) were, therefore, measured in HBO-exposed volunteers to search for shifts in extracellular fluid distribution. Lipid peroxidation was estimated by measuring plasma thiobarbituric acid-reactive substances (TBARS) in plasma as well as urine chemiluminescence (CHL). Oxidative stress resulting from HBO was further investigated by administering high doses of the antioxidant vitamins C and E.

**METHODS**

Ten healthy male volunteers (30±6 years) were exposed for one hour daily to 240 kPa ambient pressure while breathing 100% O₂. For HBO application up to four volunteers entered a Starmed 2000/5,5 hyperbaric chamber (Haux, Karlsbad-Ittersbach) which was air pressurized within two min to 240 kPa. The volunteers were then provided with 100% oxygen supplied from external flasks via tight-fitting oronasal masks. Expired air was conducted through a valve to the outside of the chamber. After one hour, the chamber was decompressed within two min down to atmospheric pressure, and the O₂ administration terminated. During HBO application the contact with the volunteers was maintained by phone and video. Temperature and total pressure, as well as O₂ and CO₂ concentrations inside the chamber were continuously monitored. After completing a five-day series of HBO exposure (series A), the volunteers were supplemented for 18 days with vitamin C and E in oral doses of 3g/die, respectively. During the final four days of antioxidant vitamin supplementation, HBO was applied again as above (series B), the subjects acting as their own controls.

Blood (antecubital vein puncture) and urine samples were collected immediately before and after each HBO application. Heparinized blood was used for the determination of RBC, Hb and Hct by standard methods (Coulter Counter Electronics, Krefeld). In serum obtained from untreated blood samples, TPC and bilirubin (Br) concentration were assayed by the Biuret and the diazonium method, respectively, using an Hitachi Boehringer Mannheim 704 Automatic Analyzer. Plasma haptoglobin concentration (Hp) was determined by using NOR-Partigen-Immunodiffusion plates (Behringwerke, Marburg). Plasma separated by centrifugation of EDTA treated blood (3000 rpm, 10 min) was stored at -80°C for later assessment of lipid peroxidation by measuring TBARS in thawed samples with a Shimadzu RF 540 spectrofluorimeter (Yagi, 1976), the results being expressed in malondialdehyde (MDA) equivalents.

Degradation of lipid peroxidation products is accompanied by light emission (Boveris et al., 1980). Lipid peroxide levels in urine were, therefore, estimated by measurement of CHL according to Lissi (personal communication). Immediately after being collected, urine samples were gassed with N₂ for 2 min and stored at -80°C until further processing. Prior to measurement the urine was thawed, titrated to pH 6 and diluted with aqua dest. to an extinction of 0.2. The titrated and diluted urine was subjected to photon counting for light emission in a Biolumat 9500 chemiluminometer (Berthold, Wildbad), the results being standardized for creatinin concentration (Boehringer Mannheim). Results from samples obtained immediately before and after each HBO application were averaged to yield individual values. Data are reported as means±SE. For analysis of differences Student’s paired t test was applied. Statistical significance was defined as p<0.05.