Alterations of Fibrinolytic Activity in Human During and After Hyperbaric Oxygen Exposure

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Abstract. To clarify the stage of fibrinolytic activation by hyperbaric oxygen (HBO) exposure, we examined its alterations in human during and after the HBO exposure. Eight healthy female volunteers breathed oxygen at 284 kPa (2.8 atmospheres absolute). Blood samples were collected before compression, shortly after compression to the pressure 284 kPa, shortly before the start of decompression, shortly after decompression, and then again 3 hours after decompression. We estimated the euglobulin fibrinolytic activity (EFA) and, the activities and antigens of both tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1). The PAI-1 activity and PAI-1 antigen showed significant decrease after compression to a pressure 284 kPa, before the start of decompression, and after decompression. The EFA level and t-PA activity rose significantly shortly after decompression, and 3 hours later returned on baseline. These findings suggest that fibrinolytic activity is elicited after HBO rather than during HBO.

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Keywords: tissue-type plasminogen activator, plasminogen activator inhibitor-1, hyperbaric oxygen

Introduction

There have been few reports about effects of fibrinolytic activity by hyperbaric oxygen (HBO) exposure. Philip et al. (1972) indicated that activations of fibrinolytic and coagulation systems were evident in divers who breathed the air at 1,013 kPa. The phenomenon was due to bubble generation in the blood. On the other hand, Phillips et al. (1992) demonstrated that oxygen exposure resulted in elevated plasminogen activator activity of cultured endothelial cells. Their data suggest that hyperoxia

induces fibrinolytic activation. Therefore, we examined alteration of the fibrinolytic activity during and after HBO exposure without the findings of bubble generation.

Materials and Methods

Subjects

Eight healthy female volunteers (aged 20 and 21 years) participated in the study. All the samples revealed no abnormalities in hematological and coagulant examinations, and without a catamenial period.

Experimental trials for HBO exposure and control

Group of 284 kPa (2.8 atmospheres absolute) oxygen inhalation: For HBO exposure, subjects were exposed for 70 minutes (from 10:10 to 11:20) to breathing oxygen at a pressure of 284 kPa in a hyperbaric chamber (Nakamura-Tekko, Tokyo, Japan). Subjects breathed oxygen using a Laerdal mask with air breaks of 5 minutes between each 20 minute period. They were decompressed over a period of 30 minutes breathing oxygen without decompression stops. Calmness was maintained to avoid mental stress during the experiment.

Control group: For atmospheric pressure air inhalation, eight subjects breathed room air during the examination period, and they used a mask to breathe air like the group for HBO exposure from 10:00 to 11:50.

Samples for fibrinolytic activities and coagulation factors

Blood was collected from an antecubital vein without using a tourniquet. The blood samples were collected before compression (9:50), shortly after compression to the pressure 284 kPa, shortly before the start of decompression, shortly after decompression, and then again 3 hours after decompression (14:50). The samples which were obtained at 284 kPa were immediately decompressed at

the level of atmospheric pressure within 1 minute (We had been certain that this rapid decompression would not affect the measurements in a previous experiment).

The sample for fibrin/fibrinogen degradation products (FDP) were collected using plastic syringes (5 ml), and immediately transferred to plastic tubes and kept with ice. After centrifugation for 10 minutes (3,000 rpm, 4°C), the serum was collected and aliquots were stored at -70°C.

All the samples except FDP were collected using plastic syringes (10 ml), containing 3.8% sodium citrate (9:1 vol/vol), and immediately transferred to plastic tubes and kept with ice. After centrifugation for 15 minutes (3,000 rpm, 4°C), the plasma was collected and aliquots were stored at -70°C. The euglobulin fractions were prepared by titration of 20-fold diluted (with distilled water) plasma with 0.25% (vol/vol) acetic acid to pH of 5.2. Following precipitation by centrifugation, the euglobulins were dissolved in a buffer containing 0.137 mol/l barbital sodium and 0.063 mol/l NaCl adjusted to pH 7.38 with 0.1 mol/l NaOH. The euglobulin fractions were stored at -70°C.

Assay methods for fibrinolytic activities and coagulation factors

Euglobulin Fibrinolytic Activity (EFA): EFA was determined by both the plasminogen-rich fibrin plate method of Astrup and Müllertz (1952) and the plasminogen-free fibrin plate method of Matsuda et al. (1972). Plasminogen-poor fibrinogen was prepared by passing bovine fibrinogen (Miles Inc., Kankakee, Ill., USA) through a Lys-Sepharose column (Pharmacia AB, Uppsala, Swe-

den). The EFA is expressed as the lysis area (mm²) on the fibrin plate.

Tissue-type Plasminogen Activator (t-PA) activity: The t-PA activity was assayed by a Tint Elize tPA kit (Biopool Inc., Umea, Sweden).

Plasminogen Activator Inhibitor-1 (PAI-1) activity: The PAI-1 activity was assayed by an Imulyse PAI-1 kit (Biopool Inc., Umea, Sweden).

t-PA antigen, PAI-1 antigen: Both the t-PA antigen and PAI-1 antigen were measured with a Spectrolyse/fibrin kit (Biopool Inc., Umea, Sweden).

Others: FDP, thrombin-antithrombin complexes (TAT complexes), and coagulation factor XII activity were determined by the latex photometric immunoassay, the enzyme immunoassay, and the chromogenic activated partial thromboplastin time method respectively, which were examined by SRL Inc., Tokyo, Japan.

Data analysis

The level of significance between different groups was determined by two-way analysis of variance (ANOVA). Student's t-test was employed when comparisons were made with the values before HBO exposure. Data are presented as the mean $\pm$ SD. Significance was taken at $p < 0.05$.

Results

As shown in Fig. 1, EFA rose significantly shortly after decompression (vs. before compression) and reverted

Fig. 1 Effect of HBO exposure on EFA. Significant difference (* : $p < 0.05$) when compared with the values before HBO exposure.

Fig. 2 Effect of HBO exposure on t-PA activity. Significant difference (* : $p < 0.05$) when compared with the values before HBO exposure.

to the pre-compression level after 3 hours from HBO exposure. The t-PA activity was reversed shortly after decompression when it rose significantly (vs. before compression), following a similar pattern to the EFA level, as demonstrated in Fig. 2. On the other hand, the PAI-1 activity showed a significant decrease during pressure held at 284 kPa and shortly after decompression (Fig. 3). The PAI-1 antigen presented a decrease during HBO exposure, following a similar pattern to the PAI-1 activity, as demonstrated in Fig. 4. HBO did not influence the t-PA antigen (before compression, $4.9 \pm 1.2 \mu\text{g/l}$; after compression, $4.7 \pm 1.4 \mu\text{g/l}$).

There was no alteration of other fibrinolysis and coagulant factors such as FDP, TAT complexes, and coagulation factor XII activity under HBO exposure.

Discussion

Fibrinolytic potential is represented by PAs and PAIs. t-PA binds avidly to fibrin and appears to be the predominant human PA involved in intravascular fibrinolysis (Collen D, 1980). In our study, an increase in t-PA activity was elicited after HBO exposure, and a decrease in activity and antigen of PAI-1 was continuous during and after exposure. We consider that alteration of both t-PA and PAI-1 have been responsible for the increase in EFA shortly after decompression.

On the other hand, in our experiment, there was a decrease in PAI-1 activity and antigen while there being not an increase in t-PA activity and antigen during HBO exposure. It has been reported that free radical derived oxidants reflected a decrease in PAI-1 activity in vitro

(Stief et al., 1990; Lawrence and Loskutoff, 1986). PAI-1 belongs to the protease inhibitors of the serpin family. It has been shown that members of the serpin family was easily inactivated by oxidation. Narkowicz et al. (1993) found that hyperbaric oxygen at 274 kPa (2.7 atm) increased the free radical levels in the blood. In our experiment, it is possible that a decrease in PAI-1 was associated with free radical. Moreover, the t-PA antigen did not vary, because of the abundant inactive t-PA compared to active t-PA in the plasma, although t-PA would continue to be released.

On the contrary, it has been reported that free radical derived oxidants reflected an increase in t-PA activity in vitro (Stief et al., 1989). Mano et al. (1994) presented that an increase of hydroxyl radicals was observed after HBO exposure rather than during HBO exposure in rabbits. In our study, it is possible that an increase in t-PA after HBO exposure was affected by a large amount of free radicals.

We have conducted the examinations on decompression bubbles by the ultrasonic tomography method (Spencer and Campbell, 1968) for 3 hours (from 11:50 to 14:50); however, no bubble was detected. Moreover, the scheme of HBO exposure that we adopted was similar to that used in therapy (U.S. Navy Diving Manual, 1985). We deduce that either an increase in t-PA or a decrease in PAI-1 was not caused by gas embolism or bubbles in blood.

Whalen et al. (1965) stated that 308 kPa oxygen inhalation led to an increase in peripheral resistance. We examined the autonomic nervous system using the method by Kunitake and Ishiko (1992), demonstrating a

Fig. 3 Effect of HBO exposure on PAI-1 activity. Significant difference (*: $p < 0.05$, **: $p < 0.01$) when compared with the values before HBO exposure.

Fig. 4 Effect of HBO exposure on PAI-1 antigen. Significant difference (*: $p < 0.05$, **: $p < 0.01$) when compared with the values before HBO exposure.

mild dominance of the parasympathetic nervous system. There is a possibility that contraction or dilatation of the peripheral vessels participate in the regulation of fibrinolytic activities.

In conclusion, we suggest that fibrinolytic activity is elicited after HBO exposure rather than during HBO exposure. Moreover, it is necessary to study using hyperbaric exposure with breathing mixed gas like nitrox or trimix with 21 kPa oxygen, because it is obvious that alterations of fibrinolytic activity are due to either HBO or hyperbaric pressure.

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