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WPŁYW NURKOWANIA NA PAI – 1 I ALFA2-ANTYPLAZMINY ORAZ AKTYWNOŚĆ FIBRYNOLITYCZNĄ

Praca mówi o nie zbadanych dotychczas dokładnie powiązaniach choroby dekompresyjnej z zagadnieniami hemostazy a szczególnie fibrynolizy. Badany był wpływ ekspozycji hiperbarycznej na główne składniki systemu fibrynolizy. Dwie grupy młodych mężczyzn poddawane były ekspozycjom hierbarycznym do ciśnienia 400 kPa – grupa I – i 700 kPa – grupa II. Stosowano dekompresje powietrzną. Po ekspozycji badano nurków na obecność objawów choroby dekompresyjnej, a także poszukiwano pęcherzyków gazu w naczyniach żylnych metodą Dopplera. 15 minut po zakończeniu dekompresji pobierano krew do badań koagulologicznych. Badano stężenie i aktywność t-PA i PAI-1, stężenie PAP i alfa2-antyplazminy. W grupach badawczych nie stwierdzono wykładników choroby dekompresyjnej ani nadmiernej ilości pęcherzyków wewnątrznaczyniowych. Stwierdzono między innymi spadek poziomu alfa2-antyplazminy, spadek stężenia i aktuwności PAI-1. Nie zaobserwowano zmian w zakresie czynnika XII jak i t-PA. Ekspozycja hiperbaryczna i dekompresja indukuje fibrynolizę, nawet bez obecności pęcherzyków gazowych.

słowa kluczowe: *alfa2-antyplazmina, choroba dekompresyjna, PAI-1, PAP, t-PA*

DECREASED LEVELS OF PAI-1 AND ALPHA2-ANTIPLASMIN CONTRIBUTE TO ENHANCED FIBRYNOLITIC ACTIVITY IN DIVERS.

There are a number of reported cases of decompression sickness (DCS) with haemorrhages. These cases have not been sufficiently investigated and thus bleeding complications could not be directly correlated to the enhanced fibrinolysis. The effect of hyperbaric exposition and decompression on the main components of fibrynolitic system have been measured. Two groups of 25 male divers each, were subjected to hyperbaric exposures to the pressure of either 400 kPa – group I – or 700 kPa – group II followed by a staged decompression. The divers were monitored for clinical symptoms of DCS and checked for Doppler-detected venous gas bubbles. Venous blood was drawn from divers before exposition and 15 minutes after decompression. The concentrations and activities of t-PA and PAI-1 as well as concentrations of PAP and alpha2-antiplasmin and activity of factor XIIa were measured. In all groups of divers no cases of DCS as well as detectable gas bubbles were noted. We observed elevated concentration of PAP, decreased concentration of alpha2-AP, decreased PAI-1concentration and activity. There were no significant changes in factor XIIa activity as well a of t- PA concentration and activity. Hyperbaric exposition and decompression induce activation of fibrynolisis, even in the absence of detectable gas bubbles. Fibrynolitic activity increases mainly due to decrease of PAI-1 concentration and activity. Further clinical trials are necessary for the estimation of the importance of activation of fibrynolisis with decreased level of PAI-1 and alpha2-AP as possible risk factor for bleeding in divers.

keywords*: alpha2-antiplasmin, decompression sickness, PAI-1, PAP, t-PA*

INTRODUCTION

It is generally accepted that decompression sickness (DCS) in divers and caisson workers is caused by gas microbubbles which are formed in venous system in nearly all decompressions [1]. The symptoms of DCS are ranging in severity from skin rush and limb or joint pain, to central nervous system disturbances, respiratory difficulties, paralysis and circulatory shock, depending presumably upon the extent and location of the offending bubbles. The quantity of bubbles observed correlates well with the severity or stress of decompression [2]. The consequence of gas microbubbles, is underrecognized and frequently overlooked. Bubbles may be trapped in the tissues and capillaries block blood flow, impair CO2 elimination and O2 uptake and lead to platelet activation and endothelial damage [3-5]. The exact mechanism for endothelial injury is also not clear. Besides its mechanical damage by the gas bubbles, activation of leukocytes and the release of oxygen radicals may also affect endothelial cells [6-8]. Known anticoagulant effects of endothelium, which are presumably suppressed after injury, platelet activation and the report of Boussuges et al. suggesting activation of coagulation in divers with neurological symptoms of DCS primary focused our interest on the possible activation of coagulation after diving and decompression [9]. In our previous study we did not observed significant changes in concentrations of prothrombin fragment 1+2, thrombin-antithrombin complex as well as d-dimer. Surprisingly we detected statistically significant increased concentrations of plasmin-antiplasmin complex in divers after hyperbaric exposition and decompression [10].

There are a number of reported cases of DCS in which nontraumatic haemorrhages into inner and middle ear, cerebellum, spinal cord, lungs, orbit subperiosteum or from existing varices have been observed in physical examination or in autopsy of divers after diving accidents [11-18]. The bleeding complications implicated in the pathogenesis of the reported cases of DCS have not been sufficiently investigated and thus could not be directly correlated to the enhanced fibrinolysis.

The aim of the present study was to measure the effect of hyperbaric exposition and decompression on the main components of the fibrynolitic system: t-PA, PAI-1, alpha2-antiplasmin, factor XII and PAP. We expected to obtain more data to clarify the possible mechanism of the enhanced fibrinolytic activity in divers.

MATERIAL AND METHODS

Two groups of 25 healthy male divers each, aged 18-40 years, who had not taken any drugs for at least two weeks prior to blood sampling, were subjected to short term hyperbaric exposures to the pressure of either 400 kPa – group I – or 700 kPa – group II – with 30 minutes bottom time followed by a staged decompression. The water depth of 60 m represents the lower limit for dives with air as a breathing medium.

The exposures were carried out in decompression habitat DGKN-120 at the Department of Diving and Underwater Work Technology, Naval Academy in Gdynia, Poland. Only air was breathed during these exposures. The decompressions were carried out according to the Polish Navy Decompressions Tables. To assure maximum safety of the study and to minimize the risk of adverse events, for divers subjected to 400 kPa decompression profile for dives to 33 m (440 kPa) was applied, and for divers subjected to 700 kPa decompression profile for dives to 63 m (735 kPa) was applied. The details of performed hyperbaric expositions and the decompression profiles of presents table 1. The divers were monitored for clinical symptoms of DCS and checked for Doppler-detected venous

gas bubbles as a risk factor for DCS. The experiments were approved by the Human Research Ethics Committee of the Medical University of Bydgoszcz.

Table 1.

Time/pressure parameters of the hyperbaric expositions and details of the decompression profiles (min = minutes)

	Number of divers	Pressure (kPa)	Corresponding water depth (m)	Exposition time at the storage depth (min)	Decompression time from the storage depth to the first stop (min)	Depth of the decompression stops (m) / Stop times (min)	Total decompression time (min)
Group I	25	400	30	30	3	9 / 6 — 6 / 10 — 3 / 16	35
Group II	25	700	60	30	6	24 / 9 — 21 / 13 — 18 / 16 — 15 / 18 — 12 / 22 — 9 / 32 — 6 / 47 — 3 / 58	221

BLOOD COLLECTION AND PREPARATION OF PLATELET POOR PLASMA

Venous blood was drawn without stasis from divers before exposition and 15 minutes after decompression, into tubes containing sodium citrate (0.32 % final concentration) (Vacutainer™, Becton Dickinson, UK) or into tubes containing citrate buffer (0.5 M, pH 4.3, StabilyteTM, Biopool, Ireland). The first 4 ml of blood were discarded. Platelet poor plasma was prepared by centrifugation at 2000 x g for 10 minutes of whole blood.

PLATELET COUNT AND HAEMATOCRIT

Platelet count and haematocrit were measured in whole blood using automated analyzer (Cell-Dyn 1600, Abbott, USA).

T-PA, PAI-1 AND PAP

The concentration and activity of t-PA were measured using t-PA Combi Actibind ELISA Kit, (Technoclone, Austria). PAI-1 concentration and activity were measured using PAI-1 Antigen ELISA, and PAI-1 Actibind ELISA (Technoclone, Austria) respectively. PAP concentration was measured using Enzygnost PAP micro kit (DadeBehring, Germany).

FACTOR XII ACTIVITY

Coagulation Factor XII Deficient Plasma (human) (Dade Behring, Germany) and activated partial thromboplastin time assay has been applied for the measurement of factor XII activity. The results were expressed in % of the norm.

ALPHA2-ANTIPLASMIN

The concentration of alpha2-antiplasmin was measured using Unitest .2AP (Unicorn Diagnostics, Great Britain).

STATISTICS

The data are presented as mean values ± standard deviation (SD). For statistical evaluation of the results Wilcoxon's test was used.

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RESULTS

All participants completed the study according to the protocol and no adverse events, no cases of DCS as well as detectable gas bubbles were noted. Haematocrit did not differ before exposition and after decompression in both groups of divers. We observed elevated, statistically significant concentration of PAP (from 60.35±25.4 to 69.18±28.6 ng/mL, $p<0.05$ – in group I and from 57.19±18.05 to 76.75±43.06 ng/mL, $p<0.05$ - in group II) (fig.1), decreased concentration of alpha2-AP (from 1.06±0.23 to 0.64±0.19 U/mL, $p<0.05$ – in group I and from 1.06±0.25 to 0.94±0.9 U/mL, $p<0.05$ – in group II)(fig.2), decreased PAI-1 concentration (from 3.92±2.05 to 2.75±2.56 ng/mL, $p<0.01$ – in group I and from 5.14±3.28 to 2.68±2.0 ng/mL, $p<0.001$ – in group II)(fig.3) and activity (from 8.57±4.88 to 5.67±2.99 AU/mL, $p<0.001$ – in group I and from 10.36±8.03 to 5.56±2.63 AU/mL, $p<0.001$ – in group II)(fig.4). There were no significant changes in t-PA concentration (from 9.46±5.59 to 8.45±4.68 ng/mL, $p>0.05$ in group I and from 9.12±5.01 to 8.84±4.75 ng/mL, $p>0.05$ – in group II). We observed increased factor XIIa activity (from 120±33.3 to 124±42.8 %, $p>0.05$ – in group I and from 110±31.2 to 130±48.5 %, $p>0.05$ in group II), however the difference did not reach statistical significance. We detect no t-PA activity in all investigated divers. According to the method applied in the t-PA Combi Actibind ELISA Kit, t-PA activity of approximately 0 U/mL should be expected in normal plasma.

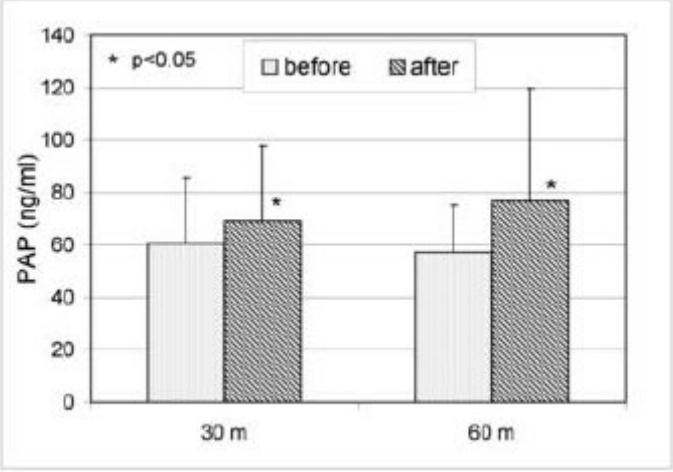

Fig. 1. The effect of diving and decompression on PAP concentration

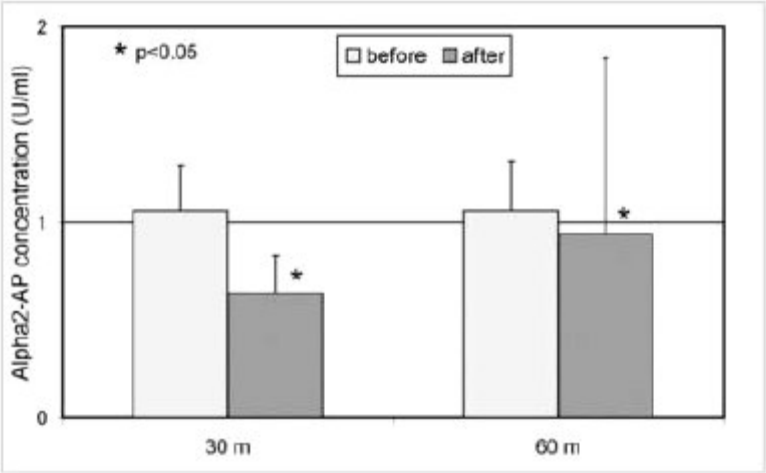

Fig. 2. The effect of diving and decompression on alpha$_2$ – AP concentration

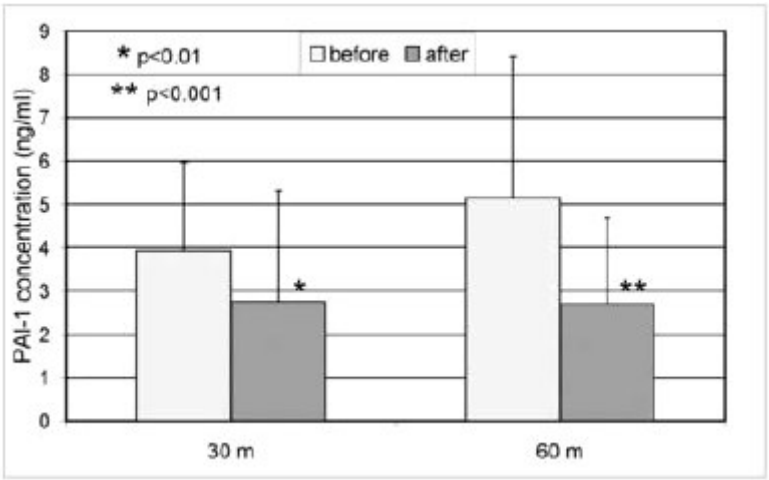

Fig. 3. The effect of diving and decompression on PAI – 1 concentration

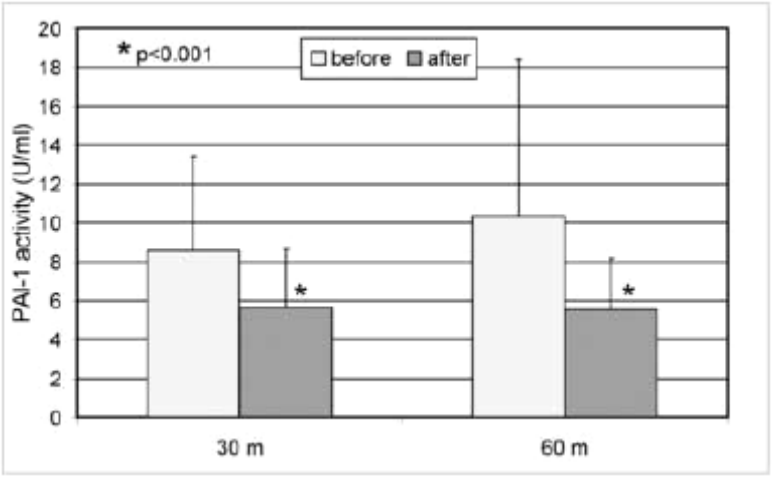

Fig. 4. The efefect of diving and decompression on PAI – 1 activity

DISCUSSION

Gas microbubbles occurring in divers' circulation after surfacing without symptoms of decompression sickness are called as "silent bubbles". Are they really silent ? They may lodge in the microvessels of various organs, causing local reactions. The knowledge of the consequences of air microemboli is very limited. Microbubbles trapped in capillaries evoke compression against endothelial cells, causing increase of large-pore radii [19] and gap formation [20]. Gap formation and hydrostatic pressure promote fluid passage into the interstitium result local edema.

Additionally to the mechanical tissue injury microbubble-induced inflammatory response may be observed [21,22]. Neutrophiles aggregate around the microbubble and release free radicals and proteolytic enzymes. The released material may lead to the interstitial pulmonary edema [23]. The air-plasma interface of microbubbles activates or serve as activating template for several proteins.

Lee and Hairstone, and Ward et al reported activation of complement system [24,25]. There was observed correlation between C3a and C5a concentrations and the incidence of decompression sickness [26]. These components of complement system present another factor that triggers neutrophiles and stimulates release reaction. The interface surrounding gas bubbles occurring in circulating blood acts as a foreign substance, which affects haemostatic system. It has been proven that platelets adhere to the bubbles resulting platelet activation and aggregation [27,28]. Electron microscopic findings indicate that induction of platelet aggregation by N2 bubbles is initiated by adhesion to the bubble wall of plasma proteins and lipids [5]. The plasma-bubble interface may activate contact factors, which play an important role in the activation of fibrinolysis. We did observed increase of plasmin-antiplasmin complex concentration [29].

In the present study divers were subjected to the hyperbaric expositions within safe table limits.They revealed no detectable gas bubbles. It is obvious that ultrasound technology used for detection of air bubbles, the only current aid, has its limits. The lack of detection of microbubbles does not exclude that they are not present.

In investigated divers we observed elevated concentration of PAP and decreased concentration of alpha2-AP after decompression. In our opinion factor XIIa presents the

most probable activator of 8plasminogen. We did measured increased activity of XIIa after decompression however the difference did not reach statistical significance. The complex formation of alpha2-AP with generated plasmin is the process resulting consumption of alpha2-AP and lowering its plasma concentration. Surprisingly, the divers showed decreased concentration and activity of PAI-1 after decompression without significant changes in t-PA concentration and activity. Decreased level of PAI-1 may contribute to enhanced fibrinolytic activity in divers. PAI-1 decrease in divers' circulation may cause imbalance in physiologic dynamic equilibrium between PAI-1 and t-PA. In that situation normal t-PA level might evoke increased fibrynolitic activity. We can only speculate which mechanism is responsible for the PAI-1 changes. It is rather less probable that this inhibitor is cleaved by generated plasmin. Platelets contribute significantly to the PAI-1 plasma concentration [30]. Their activation observed in divers should rather evoke increase than lead to the decrease in PAI-1 concentration and activity. Thus it seems to be less probable that activation and loss of circulating platelets occurring after decompression may be the explanation for the observed changes of PAI-1 level. Neutrophiles may also be considered to have a significant contribution to fibrinolysis. In fact our previous study showed changes in white cell count in divers [31]. Decreased platelet number and increased neutrophil count were observed after expositions to the pressure of 700 kPa with 35 min plateau. Two enzymes released from polymorphonuclear leukocytes: cathepsin G and elastase were identified as the major fibrinolytic enzymes [32]. They can be released directly onto the fibrin surface and are capable to cleave plasminogen, alpha2-AP and PAI-1 molecules [33-35]. Plasminogen, after limited proteolysis by catepsin G or elastase is more readily activated by plasminogen activators whereas PAI-1 and alpha2-AP loose their activity [33-35].

The observed changes in components of fibrynolitic system occurred after exposition to the pressure of 400 kPa as well as to the pressure of 700 kPa were not statistically significant different between the groups. We suppose that the most important factor responsible for observed changes in fibrynolitic system presents formation of microbubbles. The amount of microbubbles formed 9 during decompression is a function of the rate of reduction in barometric pressure and the rate at which the dissolved during hyperbaric exposition gas can diffuse across the various membrane barriers of the body and be eliminated in the expired air. If the reduction of the pressure exceeds the rate of gas diffusion a state of supersaturation occurs and bubbles may form. The time and the pressure of the exposition have only effect on the amount of gas dissolved in body fluids. The applied decompression parameters in our study were different in the groups, adjusted to the exposition pressure according to the decompression tables. The lack of significant differences between the groups may be indirect prove of the comparable safety level offered by the used decompression tables.

The measurement of PAP, alpha2-AP concentrations and PAI-1 concentration and activity seems to be more sensitive tool for detection of the effect of diving on human homeostasis than Doppler ultrasound. The hyperbaric expositions and decompression were performed with a relatively wide margin of safety in terms of DCS. However there were detected statistically significant changes in fibrynolitic system. No cases of DCS as well as no abnormal bleeding observed in investigated groups of divers unable the exact estimation of clinical importance of detected changes. It seems to be less probable that activation of fibrinolysis and decreased level of PAI-1 detected in our study may significantly increase the risk of spontaneous bleeding, thus it might contribute to the prolonged bleeding after injury. Further clinical trials are necessary for the estimation of

the importance of activation of fibrynolisis with decreased level of PAI-1 and alpha2-AP as a possible risk factor for bleeding in divers.

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