Chronic hyperbaric exposure activates proinflammatory mediators in humans

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Decompression illness (DCI) is traditionally believed to be caused by liberation of gas from tissue depots during decompression (10). It affects divers who are exposed to rapid reductions in ambient pressure in which, after decompression, gas bubbles appear in the blood (5, 7, 11, 12, 42). These bubbles are thought to act as mechanical obstructers of blood flow and thus produce symptoms of DCI. However, the symptomatology of DCI is heterogeneous and not entirely explained by a mechanical mechanism (18a). Distal symptoms are frequently seen, but many central organ systems, including the central nervous system and the respiratory system, may be affected as well (18a, 24). It is quite possible that the pathogenesis of DCI, at least in part, may be of inflammatory origin because the interindividual susceptibility to decompression trauma is highly variable and because repetitive dives have resulted in an induction of tolerance and acclimatization (3, 13, 25, 50). The delay between the decompression trauma and onset of symptoms also suggests a nonmechanical mechanism of DCI (48). Furthermore, “pre-tuning” of the inflammatory system with foreign protein has been shown to result in decreased incidence of DCI in rats (33). An inflammatory reaction as the cause of DCI has been suggested in earlier publications from different groups of investigators (25, 51, 52), and our laboratory has in an earlier study (18) been able to show an increase in interleukin (IL)-6 after severe decompression trauma in Wistar rats. The cause of an induction of an inflammatory reaction may well be the nitrogen bubbles that appear in the blood during decompression, since the surface of the bubbles can function as a blood/artificial-surface interface with a potential to trigger the activation of inflammatory cascades. Such an effect after a diving-induced blood-gas interface on inflammatory and hemostatic systems has previously been reported by several authors (8, 9). It is also known that a blood/membrane interface during cardiopulmonary bypass is capable of increasing plasma levels of neutrophil gelatinase-associated lipocalcin (NGAL) and IL-8 (29, 46, 53).

During an inflammatory reaction, anti-inflammatory substances are released to counteract the inflammatory activators. Among these different anti-inflammatory substances, IL-1 receptor antagonist (IL-1ra) is interesting because it is produced by neutrophils (26, 41) and has the capacity to competitively block the receptor of one of the proximal inflammatory cytokines (1). Promising results have recently been achieved with IL-1ra in the treatment of sepsis (43). Because activation of leukocytes has a central role in inflammation, another interesting anti-inflammatory substance is secretory leukocyte protease inhibitor (SLPI), which is produced by different cell types, including neutrophils (40, 45, 54, 56). It is a substance that has been extensively studied in experiments concerning endotoxic lipopolysaccharide (LPS) (16) and acquired immunodeficiency syndrome (38).

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In this study, we addressed the hypothesis that diving per se gives rise to an inflammatory reaction. Because diving within table limits rarely causes DCI, and knowing that repeated dives may result in acclimatization, we hypothesized that anti-inflammatory substances might be released in concert with the pro-inflammatory substances, thus keeping homeostasis intact. Pro- and anti-inflammatory activation levels, as measured by IL-8, IL-6, and NGAL (proinflammatory) and SLPI and IL-1ra (anti-inflammatory), were assayed in the systemic circulations of healthy male subjects who, during military training, were exposed to repetitive, hyperbaric exposures for prolonged periods of time.

MATERIALS AND METHODS

This study was carried out with the approval of the ethics committee of the University of Lund. All participants were informed verbally and in writing before the test period and after that gave their informed consent. All divers participated on their own free will and could choose to leave the program at any time during the trial.

Diving period. Eleven healthy male military diving trainees participated in one series in which blood samples were obtained at two occasions during a 3-mo training period. All divers were from the same contingent of military trainees at the start of the series. They had all recently passed medical and physical tests (Royal Swedish Navy medical clearance for diving personnel, SUB III) and had all received medical clearance as military divers. The divers were physically well matched with a body mass index of 22.5 ± 1.3. During the test period, they all lived in the same camp facility, ate the same food, and had identical daily schedules. During weekdays, all personnel stayed within the camp and were not exposed to any other environment. They all performed the same diving excursions and under identical environmental conditions. The hyperbaric exposure was uniformly increased during the test period to a maximum depth of 57 m of sea water (see Table 1). The amount, timing, and nature of the physical training were the same for all of the participants during the test period and did not differ from the conditions dating from 1 mo before the trial. The physical training was divided in two periods daily consisting of 1 h each.

The nature of the training was standardized according to the unit manual and was mandatory for all diving personnel. All the test subjects stayed healthy during the test period and after that gave their informed consent. All divers participated on their own free will and could choose to leave the program at any time during the trial.

Diving period. All participants completed the diving period without any medical problems. No symptoms of DCI were detected. All divers completed the full training period and did the same number of dives according to the standard diving schedule (see Table 1). Maximum excursion depth was 57 m of sea water. The total diving profile for the complete trial is shown in Table 1. In the dives in which nitrox was used, the times given for surface intervals have been set according to the actual air equivalent depth.

Predive and postdive levels of IL-6, IL-1ra, NGAL, IL-8, and SLPI were compared.

IL-6 and IL-1ra. No significant change in predive vs. postdive values was detected (P = 0.878 and 0.328, respectively) (Figs. 1 and 2).

NGAL and IL-8. Postdive values for NGAL and IL-8 increased significantly compared with baseline values (P = 0.016 and 0.026, respectively) (Figs. 3 and 4).

SLPI. Postdive values for SLPI decreased significantly compared with baseline values (P = 0.006) (Fig. 5).

The postdive developments from baseline for NGAL, IL-8, and SLPI are displayed in Fig. 6.

DISCUSSION

It is possible that the gas bubbles that appear in the circulation during decompression in diving are capable of activating immunocompetent cells and thus producing an inflammatory reaction. If this is true, some increase in inflammatory parameters in the blood of divers ought to be found even when strict diving procedures are followed, i.e., inside the limitations proposed by diving tables. In this study, we looked for changes in the blood of some inflammatory parameters

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after a long period of daily dives in a group of young healthy men under military training. We report an increase in IL-8 and NGAL and a decrease in SLPI. IL-6 and IL-1ra were also measured, but no significant changes were found.

The increase in IL-8 and NGAL is suggestive of an activation of neutrophils and probably concomitant endothelial cell activation. IL-8, which is produced by activated neutrophils (47) and activated endothelial cells (30), is a chemokine with the capacity to attract neutrophils to endothelial cells (14) and in that process

Table 1. Standard diving training program used by the military unit during the trial

<table>
<thead>
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<th>Day</th>
<th>Depth, m</th>
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<th>Surface Interval, h.min</th>
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Dec stops, decompression stops (shown as depth and minutes of stop, depth/min).

Fig. 1. Pre- and postdive values for interleukin (IL)-6. Values are given in pg/ml ± SE. NS, not significant.

Fig. 2. Pre- and postdive values for IL-1 receptor antagonist (IL-1ra). Values are given in pg/ml ± SE.
activate the endothelial cell. Activation of endothelial cells leads to production of different cytokines, including IL-8 (21). In this way, neutrophil and endothelial cell activation is propagated with a possibility to further increase the inflammatory response. It is known that IL-8 is increased in plasma after cardiopulmonary bypass (32, 40) and that neutrophils may be stimulated to produce IL-8 when adhered to a foreign surface (47). This could mean that microbubbles, preferably in the venous circulation, may be able to induce IL-8 production by producing the foreign surface that neutrophils can react with, thus starting a reaction that ultimately could lead to endothelial cell damage. It is also known that hypoxia is capable of inducing IL-8 production in endothelial cells (31). This could mean that gas embolization in the microcirculation, with ensuing substrate failure, may trigger IL-8 production and thus initiate the inflammatory cascade. The increase in NGAL that is thought to be an indicator of neutrophil activation (2) further strengthens the notion that an activation of neutrophils was at hand and is consistent with an activation of the inflammatory system.

NGAL belongs to the lipocalin protein family and is released from the neutrophil secretory granules on stimulation. The precise nature of NGAL activity is unclear, but it has been speculated that it has the capacity to bind small lipophilic inflammatory mediators (2). An increase in NGAL follows a variety of inflammatory conditions. NGAL is reported to be increased in sera and/or plasma of patients with Sjögren syndrome, emphysema, and peritonitis and in hypertensive women, as well as during extracorporeal circulation (2, 17, 29, 37). It is also increased in the synovial fluids of patients with rheumatoid arthritis (4).

SLPI has anti-inflammatory effects. Production of SLPI is known to be stimulated by LPS, IL-6, and IL-10 (27, 28). SLPI is reported to inhibit macrophage uptake of LPS (16), decrease production of inflammatory mediators (tumor necrosis factor-α, prostaglandin E2, and prostaglandin H synthase-2) by macrophages (36, 56), and initiate production of anti-inflammatory cytokines, such as IL-10 and transforming growth factor-β (45) by macrophages. Furthermore, SLPI reduces hepatocellular injury after hepatic ischemia (36), is increased during severe sepsis (19), and is reported to inhibit experimentally induced inflammatory lung injury (35). SLPI is also known to have bactericidal effects (22, 23) and has been reported to interfere with human immunodeficiency virus entry into cells (39). We found decreased levels of SLPI in the postdive period. A decrease in SLPI levels has earlier been reported during experimentally induced interaction between human immunodeficiency virus and monocytes; the notion was that SLPI was intracellularly displaced (39, 40).

Decreased levels of SLPI were also found in the initial phase of experimentally induced hepatic ischemia and likewise sepsis when no actual cell damage was probable (19, 36). During the experiments regarding experimental sepsis, it could be shown that the SLPI levels paralleled the severity. When cell damage is at hand, as in late-stage ischemia or sepsis, SLPI levels increase (19, 36). Because it is most improbable that actual cell damage should result under safe diving procedures, the decrease in SLPI levels found in this
study is in line with the induction of a low-grade inflammatory reaction.

IL-6 is one of the proinflammatory cytokines that is released during major cellular insults such as sepsis and trauma (15, 34, 44, 49). In this study, we report unchanged levels of IL-6 in contrast to what we found in an earlier study of severe decompression trauma (18). This is not surprising because the levels of IL-6 parallel the degree of trauma to the organism, with higher levels predicting a greater magnitude of cell damage (20, 49), at least in sepsis. It is quite probable that the degree of trauma in this study was too small to elicit increased IL-6 expression.

We can only hypothesize that the absence of impact on IL-1ra levels found in this study is due to the low degree of trauma that was used or that it is due to the outstretched time scale of the experiment. However, we have no hard data to support this notion.

To conclude, we found an increase in IL-8 and NGAL as a sign of neutrophil activation together with a decrease in SLPI as a sign of influence on the inflammatory defense system. No influence was found on IL-6 and IL-1ra.

Our study was designed as an observational study because a similarly well-matched control group consisting of equally fit nondivers with identical physical training and living conditions during the same period was not obtainable. Apart from the diving activity, no changes in daily routines, training, and stress were present compared with predive conditions.

Our findings are consistent with a low-grade activation of the inflammatory system with signs of an influence on the anti-inflammatory system. It is probable that the bubble formation during decompression is the trigger of this inflammatory response. It is also probable that the interplay between pro- and anti-inflammatory substances is a fine-tuned balancing act and that it is only when this balance collapses that morbidity ensues.

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