Complement activation during saturation diving


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Stevens DM, Gartner SL, Pearson RR, Flynn ET, Mink RB, Robinson DH, Dutka AJ. Complement activation during saturation diving. Undersea & Hyperbaric Medicine 1993; 20(4):279–288.—In this study, the levels of activated complement fragments C3a and C5a were measured on 11 U.S. Navy divers as they performed a 28-day saturation dive to a pressure equivalent of 1,000 feet of seawater (fsw, 31.3 atm abs). Two subjects developed symptoms consistent with the high pressure nervous syndrome (HPNS) and three were treated for type I DCS (joint pain only). These events allowed us to test two hypotheses: a) alterations in C3a or C5a levels during compression are related to the occurrence of HPNS and b) increases in complement fragments are an indicator of decompression stress associated with type I DCS. There was no correlation between changes in C3a and C5a levels during compression and the diagnosis of HPNS. Our results suggest that an increase in C3a and C5a levels during saturation diving correlates with decompression stress and the clinical diagnosis of type I DCS.

C3a, C5a, decompression sickness, high pressure nervous syndrome

Previous studies by Ward et al. (1) have shown that rabbits with plasma demonstrating a higher level of complement activation in response to in vitro exposure to gas bubbles or zymosan (a known complement activator) were more susceptible to decompression sickness (DCS) than those rabbits whose blood was unaffected by these agents. Furthermore, rabbits that were decomplemented either by multiple dives or by cobra venom injection had no symptoms of DCS while their complement levels remained low. After 1–2 days, complement levels returned to normal and the rabbits that had been made resistant to DCS were again highly susceptible after reexposure to dive profiles that produced intravascular bubbles. Thus, Ward concluded that a) complement activation could predict susceptibility to DCS, b) complement activation was a critical event in the pathology of DCS, and c) alteration in the levels of complement system components could explain the benefits of acclimatization. The discovery of cellular or biochemical processes that are inherent in the development of DCS might suggest interventions useful in the treatment or prophylaxis of this disorder.
Activation of the complement cascade results in the formation of bioactive peptides, including C3a and C5a, which may be relevant to DCS. C3a and C5a are potent anaphylatoxins that can precipitate histamine release from mast cells, initiate contraction of smooth muscle cells, and increase vascular permeability. In addition, C5a is chemotactic for neutrophils, stimulates their formation of oxygen radicals, and increases adherence to endothelial cells (2, 3). Some of the signs and symptoms of DCS mimic those of activated complement fragments including edema, pruritus, increased vascular permeability, thrombocytopenia, hemoconcentration, and leukopenia (4).

Exposure to very high pressure results in a disturbance of neurologic function referred to as the high pressure nervous syndrome (HPNS). Symptoms of HPNS typically include nausea, tremor, and myoclonic jerks. Symptoms may progress to include convulsions. We compared C3a and C5a levels during HPNS with base-line measurements to investigate whether alterations in calcium metabolism during HPNS are reflected by changes of complement activation by-products.

In this study, activation of the complement system was monitored by measuring C3a and C5a levels in 11 U.S. Navy divers as they performed a 28-day saturation dive to a pressure equivalent of 1,000 feet of seawater (fsw, 31.3 atm abs). Symptoms consistent with HPNS and DCS occurred during the course of the dives. Accordingly, an attempt was made to correlate any significant changes in the levels of the complement activation fragments during the course of the dive with these clinical diagnoses. Specifically, we tested the hypotheses that significant changes in C3a or C5a levels during compression were associated with HPNS, whereas those occurring during decompression were associated with severe decompression stress and DCS.

MATERIALS AND METHODS

A series of three saturation dives to a depth equivalent of 1,000 fsw (31.3 atm abs) were conducted in the Man-Rated Chamber Complex of the Naval Medical Research Institute (NMRI). Dives I and II had four subjects each and dive III had five subjects. No diver participated in more than one dive of this series. The protocol was approved by the NMRI Committee for Protection of Human Subjects, and divers participated only after giving their informed consent.

Dive profile

The planned profile is shown in Fig. 1. Modifications in this schedule were made for the treatment of DCS (see below). Linear compression was completed in 24 h during dive II (to maintain schedule after an operational delay) whereas 48 h was allowed for dives I and III. During the profile the divers breathed helium-oxygen mixtures with the partial pressure of oxygen maintained at 0.425 atm abs in accordance with standard U.S. Navy diving procedures (5). Decompression began on Day 17 with an upward excursion to a pressure equivalent of 849 fsw (26.7 atm abs) at a rate of 60 ft/min. No divers experienced symptoms of DCS during or within 4 h of this excursion. Decompression was continued after a 2.5-h hold at 26.7 atm abs in dives I and II, whereas in dive III the hold time was extended to 4 h 15 min
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1000 FSW saturation dive profile

[Diagram showing a dive profile with depths and oxygen concentrations over time]

FIG. 1—Dive profile and sample time points.

before commencing decompression. After completing the postexcursion hold, the decompression schedule was the same for each dive, with alterations as necessary to treat DCS.

Venous blood sampling and preparation

Forty milliliters of blood was drawn by venipuncture from the antecubital vein at seven sample (S) points along the dive profile (Fig. 1). Samples were drawn 1 wk before the dive (S1), the day of the dive (S2), within 1 h of reaching the bottom (S3), just before the excursion (S4), after the excursion (S5), upon reaching the surface (S6), and again 1 wk later (S7). The samples were collected using EDTA (Sarstedt Monovette) tubes and were placed on ice (1.2–2.0 mg EDTA/ml blood). The samples were cold centrifuged for 15 min, the plasma separated avoiding bubble formation, and placed into tubes containing fluorocarbon oil (Halocarbon oil series 56S, Halocarbon Products Corp, Hackensack, NJ) at a volume ratio of 10:1 oil to plasma. The oil was added to prevent bubble formation during decompression of the blood samples to the surface according to a technique developed at Duke University (personnel communication, Dr. R.E. Moon, Department of Anesthesiology, Duke University, March 1989). The sample and oil mixtures were placed on ice, and samples taken at depth were decompressed to the surface over 60 min (rate of 17 ft/min from 30.8 atm abs, 14 ft/min from 26.7 atm abs). At the surface the sample was separated from the oil, avoiding bubble formation, divided into 4 aliquots, and frozen at −70°C until assayed.
Complement activation assays

Products of complement activation, C3a and C5a, were assayed using an I-125 radioimmuno assay procedure for C3a, C5a, and their -desArg metabolites (Amersham Corp., Arlington Heights, IL). Samples and standards were run in duplicate, and the radioactivity present in each tube was counted for 120 s in an LKB gamma counter. The radioimmunoassays were performed in a double-blind fashion: divers did not know the result of the tests and the assays were performed by personnel without knowledge of which divers, if any, had developed DCS.

Validation tests

Two additional complement activation tests were performed to validate the techniques utilized in this study. The first assessed the reliability of the radioimmunoassay kit. A venous blood sample was drawn from one nondiver and prepared in a manner identical to that described for the diver samples. The sample was tested for a total of 32 trials, each trial performed in duplicate. The second test was to assess the effect of the compression–decompression cycle itself and the addition of fluorocarbon oil on complement activation measurements. Blood samples were drawn from two nondivers, centrifuged, and separated as described above. Each nondiver’s prepared plasma was divided into 4 aliquots, which were exposed to one of the following conditions. The 1st aliquot was tested without further manipulation. The 2d aliquot was tested after the addition of 10:1 vol of fluorocarbon oil. The 3d aliquot was subjected to a dive to 31.3 atm abs (chamber atmosphere was a 4% oxygen in helium gas mixture, the total bottom time was 15 min, and decompression to the surface required 60 min). The 4th aliquot was subjected to the same dive profile; however, while on the bottom, 10:1 vol of fluorocarbon oil was added through a chamber penetrator to the aliquot, using an external pressure pump.

Statistical analysis

A one-way analysis of variance (ANOVA) with repeated measures was used to determine if there were any significant changes in either C3a or C5a levels over the course of the dive. If a significant difference was found, a post-hoc Neuman-Keuls test was applied to determine the specific sample time points at which significant variation had occurred. The data at these time points were then divided according to the following criteria. If the significant variation occurred during the compression phase of the dive (S3, S4), the data were divided to compare the values of divers with symptoms of HPNS with those without symptoms of HPNS. If the significant variation occurred during the decompression phase of the dive (S5, S6, S7), the data were divided according to whether the diver had been treated for DCS. These comparisons were done with a Student’s t test with the Bonferroni correction. Significance was considered at $P < 0.05$. Data are presented as the mean $\pm$ SD unless otherwise indicated.

RESULTS

The C3a radioimmunoassay kit yielded consistent results (31/32 samples with agreement between duplicates had a coefficient of variation $= 7.6\%$) when one
Table 1: Validation Test No. 2*

<table>
<thead>
<tr>
<th></th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface</td>
<td>291</td>
<td>261</td>
</tr>
<tr>
<td>Surface + fluorocarbon oil</td>
<td>275</td>
<td>334</td>
</tr>
<tr>
<td>31 atm abs Dive, no fluorocarbon oil</td>
<td>275</td>
<td>248</td>
</tr>
<tr>
<td>31 atm abs Dive, fluorocarbon oil added at bottom</td>
<td>248</td>
<td>277</td>
</tr>
</tbody>
</table>

*All values relate to C3a levels in nanograms per milliliter.

sample was tested multiple times. The results of the second validation test are listed in Table 1. Adding fluorocarbon oil or exposing aliquots of the same samples to the compression–decompression profile described above did not seem to alter C3a levels.

Thirteen divers participated in this saturation dive series. We were unable to obtain samples from one of the four divers in dive 1; he did not develop symptoms of HPNS or DCS. In dive II, one of the four divers (diver 12) developed symptoms of maxillary sinusitis and required treatment with antibiotics. His C3a and C5a levels were obtained and reached a maximum of 1,959 and 41 ng/ml, respectively, at S4 during the height of his clinical symptomatology. These values were excluded from the initial statistical comparisons because bacterial infections are known to raise plasma levels of complement activation fragments (6). This diver did not develop symptoms of HPNS or DCS. After the saturation dive he was treated surgically for chronic congestion and infection of the maxillary sinuses. Thus, the initial analyses in this study utilized the data available from the remaining 11 subjects. Data were analyzed a second time with inclusion of data from diver 12.

During the dive series, the samples were recovered in good condition from depth without evidence of hemolysis or bubbling. Typically, sample preparation required 2.5 h, although the first sample at depth (S3) required up to twice as much time. After preparing the samples once at depth, the divers were able to decrease preparation time to 2.5 h at S4. During preparation the samples remained on ice.

Three of four divers in dive II developed symptoms consistent with type I DCS. The first diver to be treated had complained of pain in his left knee at a depth equivalent to 34 fsw. After recompression to 40 fsw equivalent, he reported immediate relief and was treated with four 25-min, 100% oxygen-breathing periods. The second diver awoke from sleep complaining of significant pain in both lower legs at a depth equivalent of 10 fsw. His neurologic exam was normal and he gained relief with staged recompression to 30 fsw. After five 25-min, 100% oxygen-breathing periods he had no further symptoms. The third diver with DCS complained of pain in the right knee at a depth of 10 fsw equivalent. He was recompressed along with the second diver described above. After his fourth 100% oxygen-breathing period he was asymptomatic. All treatments were completed at 0750 h of dive Day 28. At 2000 h that evening the divers were on the surface and S6 was drawn for complement assay. Thus, a blood sample was taken (S6) approximately 14.5 h after completion of treatment for type I DCS.

Manifestations of HPNS were irritability, nausea, and tremor. These symptoms were noted in two divers; one from dive II and one from dive III. The results of the
complement assays are shown in Table 2. There was a significant elevation \((P < 0.05)\) in C3a and C5a levels at S6, and C3a was also significantly elevated at S3. Although the elevation at S3 was not found to be related to HPNS \((P > 0.05)\), the elevations of C3a and C5a at S6 were highly related to DCS \((P < 0.001)\) (see Figs. 2 and 3). It should be noted that inclusion of diver 12 in the statistical comparisons did not change the correlation of type I DCS to the elevations of C3a and C5a at S6 \((P < 0.05)\). The C3a and C5a levels at S6 for divers treated for DCS were 1,941 ± 290 and 118 ± 29, respectively, whereas those for divers not treated for DCS were 217 ± 74 and 9 ± 1, respectively. When remeasured 1 wk later (ST7), the levels of C3a and C5a in divers treated for type I DCS had returned to normal.

**DISCUSSION**

Activation of the complement cascade plays a role in several pathologic conditions, and the levels of C3a and C5a reported here are within the range observed by other investigators. Hopkins et al. (7) report that levels of C3a increase to 550 ng/ml (3 times normal) during an acute exacerbation of systemic lupus erythematosus and to 1,297 ng/ml (7 times normal) in patients with lupus cerebritis. Elevation of C3a levels correlated with the severity of the pulmonary insult in intensive care patients with the adult respiratory distress syndrome \((1,105 ± 53\, \text{ng/ml})\) or severe pulmonary dysfunction \((825 ± 36\, \text{ng/ml})\) (8). Corresponding C5a levels were also elevated in these patients and were 106 ± 6 ng/ml and 55 ± 3 ng/ml, respectively. Thus, the increases in C3a to 1,941 ± 290 ng/ml and C5a to 118 ± 29 ng/ml, which were observed in this study after treatment for type I DCS, were within the ranges reported in other acute immunopathologic disease processes in which complement activation is known to occur. No similar rise in complement activation products was noted in those divers who were not treated for type I DCS.

Our data showed a marked difference in the level of complement activation between those divers who were treated for DCS and those who were not. The timing of the elevations is a puzzle. Within minutes, unbound C3a and C5a undergo secondary enzymatic degradation by serum carboxypeptidase-N (SCPN) to C3a_desArg and C5a_desArg, respectively (5). Both C5a and C5a_desArg are rapidly cleared from the circulation by binding to high affinity receptors on neutrophils (9) and platelets (10). C3a and C3a_desArg are also rapidly bound to platelets, and little remains free in the serum (11). In a primate model, C5a elevations induced by i.v. infusion of *Escherichia coli* returned to control levels in 120 min, whereas C3a levels remained elevated at 240 min (12). In our study, both C3a and C5a levels were elevated 14.5 h after successful treatment of type I DCS. This suggests that complement activation continues but is unaccompanied by symptoms. Complement activation may therefore be an epiphenomenon of DCS unrelated to symptom expression; or successful treatment reduced but did not eliminate complement activation. We would need blood samples drawn while the diver is symptomatic to decide this issue.

The kinetics of complement activation by bubbles has been addressed in part by Hjelde et al. (13). An increase in complement activation occurred primarily in the last 20 min of their 1-h in vitro study of bubbling blood plasma (13). An in vitro study by Shastri et al. (14) demonstrated increasing levels of C5a_desArg for 60 min after completion of exposure to nitrogen bubbles when compared to controls not
### Table 2: C3a and C5a Levels

<table>
<thead>
<tr>
<th>Subject (Dive No.)</th>
<th>HPNS, Y/N</th>
<th>DCS, Y/N</th>
<th>C3a, ng/ml</th>
<th>C5a, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>N</td>
<td>N</td>
<td>302</td>
<td>393</td>
</tr>
<tr>
<td>S2</td>
<td>N</td>
<td>Y</td>
<td>325</td>
<td>426</td>
</tr>
<tr>
<td>S3</td>
<td>Y</td>
<td>N</td>
<td>286</td>
<td>366</td>
</tr>
<tr>
<td>S4</td>
<td>N</td>
<td>N</td>
<td>223</td>
<td>366</td>
</tr>
<tr>
<td>S5</td>
<td>Y</td>
<td>Y</td>
<td>244</td>
<td>300</td>
</tr>
<tr>
<td>S6</td>
<td>Y</td>
<td>N</td>
<td>351</td>
<td>320</td>
</tr>
<tr>
<td>S7</td>
<td>N</td>
<td>N</td>
<td>300</td>
<td>295</td>
</tr>
<tr>
<td>S11</td>
<td>Y</td>
<td>N</td>
<td>323</td>
<td>1,282</td>
</tr>
<tr>
<td>S12</td>
<td>Y</td>
<td>N</td>
<td>216</td>
<td>298</td>
</tr>
</tbody>
</table>

*Patient with maxillary sinusitis.*
exposed to nitrogen bubbles. Venous gas embolisms (VGEs) have been shown by Doppler monitoring to occur throughout decompression from saturation dives (15). The effect of continued VGE may be responsible for the elevation in complement activation noted in our divers with DCS. The normal levels of complement activation
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measured in the divers without DCS may be due to the occurrence of less VGEs or a lack of sensitivity to complement activation by bubbles. Doppler monitoring of saturation decompression-induced VGEs while measuring complement activation might answer this question. Individual variations in sensitivity to bubble-induced complement activation has been demonstrated by Ward et al. (4) and related to the risk of DCS. Thus, complement activation may be an indicator of decompression stress in divers who are sensitive to bubble-induced activation of the complement system. We found the posttreatment elevation in complement activation products had returned to normal when remeasured 7 days later. None of the divers treated during this series of saturation dives showed any evidence of residual pain on follow-up, nor did any receive additional hyperbaric oxygen (HBO) treatments.

In this series, treatment of the divers with DCS consisted of recompression and 100% HBO breathing. In a previous study, HBO at 2.8 atm abs was shown to have no effect on the complement levels (CH50) of experienced divers (16). Studies using a guinea pig model demonstrated a small (10%), transient (< 36 h) decrease in the CH50 after exposure to HBO, indicating a degree of complement activation (17). At 48 h the complement levels (CH50) were elevated 25% from base line, returning slowly to normal over 10 days. Neither the timing nor the magnitude of these changes in complement levels are comparable to those reported in this study.

The elevation of C3a observed at S3 could have several sources. Divers with HPNS did not have unusual elevations. All of the divers reported compression arthralgias; this may be a potential source of complement activation. The sample S3 took longer to prepare than others and this might artifactually elevate complement activation products. Further studies will be necessary to determine whether compression to deep depths affects complement activation.

We find persistently elevated levels of complement fragments after successful treatment for pain-only DCS. This continued activation of the complement pathway during the last day of saturation decompression may represent either continued liberation of intravascular gas without symptomatology, or activation by lingering subclinical tissue damage after DCS. Complement activation may be an indicator of decompression stress in individuals who are “sensitive” to venous-bubble-mediated mechanisms of complement activation.

This work was supported by the Naval Medical Research and Development Command work unit no. 61153N MR04101.001-1055. The opinions and/or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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