Effect of splenectomy on platelet activation and decompression sickness outcome in a rat model of decompression

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Abstract

(Lambrechts K, Pontier J-M, Mazur A, Buzzacott P, Goanvec C, Wang Q, Theron M, Belhomme M, Guerrero F. Effect of splenectomy on platelet activation and decompression sickness outcome in a rat model of decompression. *Diving and Hyperbaric Medicine*. 2014 September;44(3):154-157.)

Introduction: Splenic platelets have been recognized to have a greater prothrombotic potential than others platelets. We studied whether platelets released by splenic contraction could influence the severity and outcome of decompression sickness (DCS) and bubble-induced platelet activation.

Methods: Sixteen, male Sprague-Dawley rats were randomly assigned to either a control or a splenectomized group. Both groups were compressed to 1,000 kPa (90 metres' sea water) for 45 min while breathing air before staged decompression (5 min at 200 kPa, 5 min at 160 kPa and 10 min at 130 kPa). The onset time of DCS symptoms and of death were recorded during a 60-min observation period post dive. Parameters measured were platelet factor 4 (PF4) for platelet activation, thiobarbituric acid reactive substances (TBARS) for oxidative stress status and Von Willebrand factor (VWf) for endothelial activation.

Results: There were no differences between the groups in DCS outcome or in PF4, TBARS and VWf concentrations.

Conclusion: These results do not support that the spleen and its exchangeable platelet pool is involved in DCS pathogenesis in a rat model, invalidating the hypothesis that increased decompression-induced platelet aggregation could be influenced by splenic contraction and then play a role in DCS outcome.

Key words

Pharmacology, platelets, physiology, treatment, decompression sickness, animal model

Introduction

Decompression from a scuba dive may result in the production of both intra- and extra-vascular bubbles. These cause a complex pathophysiological cascade that includes vascular dysfunction, microcirculatory alterations, inflammatory processes with leucocyte adhesion, pro-coagulant activity and oxidative stress, leading to decompression sickness (DCS).[1–9] Circulating bubbles are thought to affect the clotting system both through activation of the coagulation cascade and the induction of platelet aggregation. In a rat model, the post-dive decrease in platelet count (PC) correlated with severity of DCS, indicating that platelet activation and aggregation are associated with the pathogenesis of DCS.[10,11] Moreover, clopidogrel, an inhibitor of the P2Y12-receptor, reducing ADP-induced platelet activation, has been shown to reduce both DCS severity and platelet count after decompression.[12,13]

Activated endothelial cells are known to inhibit anti-coagulant mechanisms while stimulating pro-coagulant ones, by releasing substances such as tumor necrosis factor (TNF) which will induce tissue factor (TF) production, a pro-coagulant factor. Conversely, activated platelets following diving have been shown to release platelet factor 4 (PF4) in the rat and microparticles (MPs) in divers.[6,8,14] MPs generated by decompression stresses precipitate neutrophil activation, vascular damage and thus endothelial activation.[7]

In physiological conditions, the spleen contains an important number of erythrocytes, leukocytes and platelets.[15,16] About 30% of the total number of platelets are stored in an exchangeable splenic pool, with a mean platelet volume (MPV) 20% higher than the MPV of circulating platelets, owing to increased concentrations of procoagulant surface proteins (P-selectin, GP IIb/IIa).[17] Platelet size correlates positively with platelet reactivity demonstrating that platelets with higher MPV are more active haemostatically.[18]

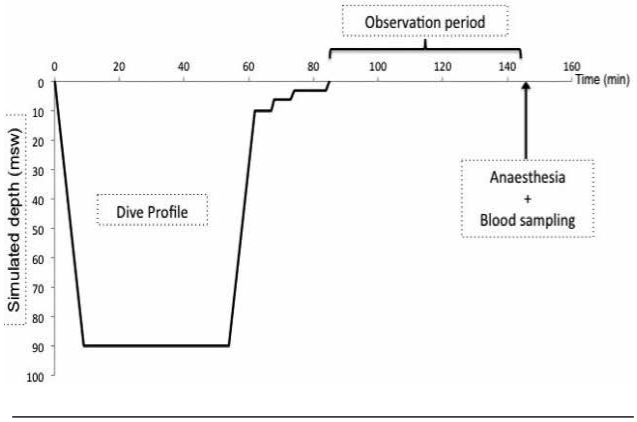
The aim of the present study was to assess whether the spleen might be involved in DCS severity, DCS outcome and platelet activation following an air-breathing compression/decompression protocol known to provoke a predictable proportion of DCS in a rat model.

Materials and methods

ANIMALS

Male Sprague-Dawley rats ($n = 16$; Janvier SAS, Le Genest St Isle, France), aged 11 weeks and weighing 376 ± 27 g (mean ± SD) were used in the study. Animals were housed two per cage, under controlled temperature (21 ± 1°C) and lighting (12 h of light, 0800–2000 h; 12 h of dark, 2000–0800 h) with access to standard rat food and water ad libitum. Rats were studied ≥ 7 days after arrival. Animal experiments were conducted in accordance with the *Guide*

Figure 1
Timeline including hyperbaric exposure profile, observation period and blood sampling



for the Care and Use of Laboratory Animals (US National Institutes of Health; NIH Publication No. 85–23, revised 1996) and with the approval of the local ethics committee for animal experimentation (approval No. 1462.01). This study accords with recognised ethical standards and national/international laws.

SURGICAL PROCEDURE FOR SPLENECTOMY

The rats were randomly assigned to one of two groups: a splenectomy group (SP, n = 8) underwent a splenectomy while a sham group underwent sham surgery (SHAM, n = 8). The rats were anaesthetized by intraperitoneal injection of ketamine (80 mg·kg^{-1}) and xylazin (15 mg·kg^{-1}), shaved and placed on an operating board and secured with tape. Midline laparotomy (3 cm) was performed under full sterile conditions. In the SP group, the spleen was identified and resected after ligature of the splenic vessels. In the SHAM group, the spleen was lifted out of the abdomen and then put directly back into the peritoneal cavity. The peritoneal cavity was irrigated with warm normal saline and the wound closed in two layers.

Individual rats were placed in separate cages postoperatively. Postoperative pain was treated with buprenorphine (Bupracare, Animalcare, Dunnington, UK) 0.3 mg·kg^{-1} injected intraperitoneally twice daily for three days. The rats were allowed to recover for two weeks after surgery before hyperbaric exposure.

DIVE PROFILE AND DECOMPRESSION PROTOCOL

Each rat was positioned in a 130-L steel hyperbaric chamber, always at the same time of day and then compressed with air at a rate of 100 kPa·min^{-1} up to 1,000 kPa absolute pressure (90 metres' sea water equivalent) and remained at this pressure for 45 min. Decompression was performed at a rate of 100 kPa·min^{-1} with three decompression stops: 5 min at 200 kPa, 5 min at 160 kPa and 10 min at 130 kPa. Total dive time was 83 min. This dive profile has previously been described and is known to reliably induce DCS in approximately 70% of rats.[6,19] For one hour after the exposure, the rats were passively observed for the appearance of signs of DCS such as unusual fatigue, ambulatory deficit, abnormal breathing, convulsions or death. The rats were classified into three categories: dead, alive with obvious symptoms within 60 minutes post dive or no symptoms of DCS (Figure 1).

BLOOD SAMPLING AND ELISA

Following the observation period, surviving rats were anaesthetized with pentobarbital (50 mg·kg^{-1}) by intraperitoneal injection. Intracardiac blood collection was performed immediately following anaesthesia or death into a BD Vacutainer® citrate tube (0.11 M) and into 2 mL Eppendorf® tubes with 30 µl 7.5% EDTA as an anticoagulant. Afterwards, surviving rats were euthanized whilst still anaesthetized by a lethal intraperitoneal injection of pentobarbital.

Blood was centrifuged at 1000 g and 4^{O}C for 10 min. Collected plasma was aliquoted and stored at -80^{O}C until assayed. The concentrations of markers of platelet activation: platelet factor 4 (PF4), endothelial activation (Von Willebrand factor, VWf), and oxidative stress status (thiobarbituric acid reactive substances, TBARS) were determined using commercially available ELISA kits for PF4 (Uscn Life Science Inc., Houston, USA), for VWf (Cusabio Biotech., Wuhan, China) and for TBARS (Cayman Chemical, Michigan, USA). Assay procedures were performed according to provider's instructions.

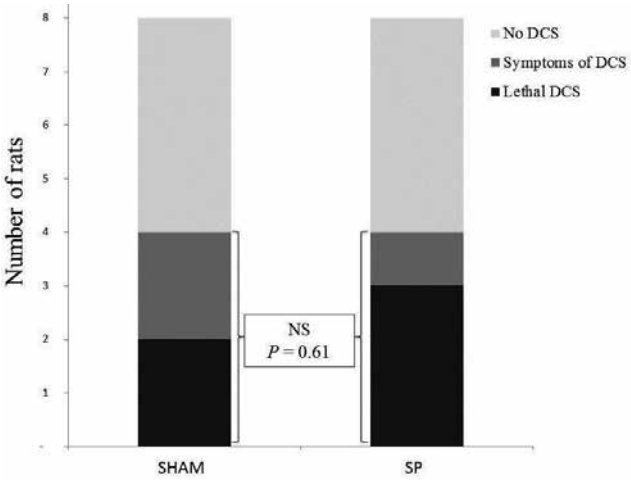
STATISTICAL ANALYSIS

Data are expressed as mean ± SD; n indicates the number of subjects in each group. For statistical analysis of blood marker concentrations post dive we used the Statistica 10 programme (Tulsa, Oklahoma, USA). Student's t-tests were used to compare groups of paired data (PF4, vWF and TBARS) after Kolmogorov-Smirnov tests for normality. Where data were not normally distributed, Wilcoxon signed rank sum tests were performed. Finally, the influence of splenectomy upon DCS was tested for significance with a Fisher's exact chi-square test. Significance in all cases was accepted at $P \leq 0.05$.

Results

The mean weights for the two groups of animals did not differ significantly (P = 0.85). There was no difference between the groups in the incidence of DCS symptoms (SHAM: n = 2, SP: n = 1). Five of the rats died within the 60-min observation period post dive. For DCS prevalence, including symptomatic and dead rats, there was no

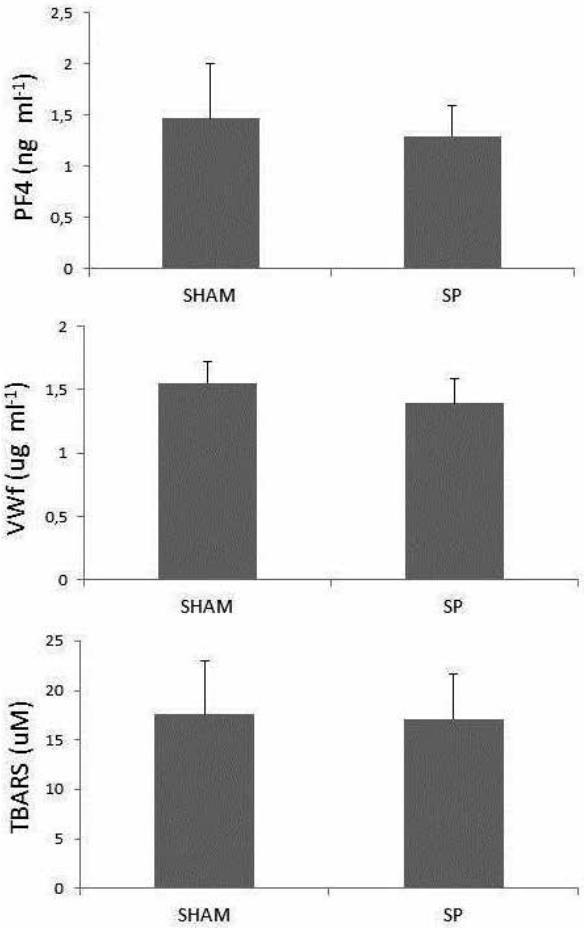
Figure 2
Number of lethal decompression sickness (DCS) (within 60 min of decompression), symptoms of DCS (paralysis or dyspnoea) or no symptoms following decompression in sham or splenectomised rats, n/s = not significant



significant difference in DCS outcome between the group ($n = 4$, $P = 0.61$). There were two deaths from DCS in the SHAM group and three in the SP ground (chi-square test $P = 0.27$; Figure 2).

Comparing plasma markers, no significant differences between the two groups were detected in any of the tests (Figure 3). Following decompression, the PF4 plasma concentration was 1.47 ± 0.54 ng·ml^{-1} in the SHAM group vs. 1.29 ± 0.31 ng·ml^{-1} in the SP group; VWf was 1.55 ± 0.17 µg·ml^{-1} in the SHAM group vs. 1.39 ± 0.19 µg·ml^{-1} in the SP group. The post-dive TBARS concentration was 7.61 ± 5.38 uM in the SHAM group compared to 17.12 ± 4.89 uM in the SP group.

Figure 3
PF4 – platelet factor (ng·ml^{-1}), VWf – Von Willebrand factor (µg·ml^{-1}) and TBARS – thiobarbituic acid reactive substances (uM) concentrations after decompression in sham rats (SHAM) and splenectomised rats (SP)



Discussion

The aim of the present study was to investigate whether the spleen and its exchangeable platelets could influence DCS outcome, using splenectomized and intact rats. We found no differences in either DCS outcome or platelet activity between the control and splenectomized groups. This suggests that the spleen does not play an important role in the pathogenesis of DCS in this model following a DCS-provoking, deep air-dive profile.

During exercise, or in aquatic mammals during diving, the spleen serves as a dynamic red cell blood reservoir.[20] Splenic contraction increasing haematocrit and haemoglobin content has been reported in diving mammals, such as the Weddell seal.[21] In humans, splenic contraction has been shown to prolong apnea dives.[20] A rapid, sustained increase of MPV in systemic venous blood, but without any changes in total platelet count, has been reported after repetitive breath-hold dives.[22] These results suggest that splenic contraction and the release of larger platelets are part of the diving response during breath-hold diving.

The results of a human breath-hold study suggest that there must be splenic capture of smaller platelets in addition to ejection of the larger ones since platelet counts were unchanged.[22] Our results demonstrate that PF4 concentrations correlate with DCS outcome, and do not change if the DCS outcome remains unchanged. Besides this, the equivalent level of oxidative stress (TBARS) and endothelial activation (VWf) between both groups is consistent with the equivalent concentration of PF4. As these three factors interact, a difference in platelet activation would have influenced the status of free radicals and endothelial cells. In the case of increased decompression stress and platelet activation we should have observed higher concentration of TBARS owing to NAD(P)H oxidase–dependent O_2 release and significant levels of VWf due to endothelial activation by stimulation of platelet MPs.[7,23] The similar levels of PF4, TBARS and VWf between groups are consistent with an equal platelet concentration and an unchanged DCS outcome.

Conclusion

This study suggests that splenic contraction, normally considered to be a physiological response to breath-hold diving, is not involved in platelet activation or DCS incidence after a DCS-provoking air dive in a rat model. However, we have not established whether this lack of effect is the result of a non-influence of large platelets released by the spleen because of decompression stress or if it is, at least partly, a result of the nonexistence of spleen contraction during diving. Further research should aim to demonstrate whether or not scuba diving induces splenic contraction in man.

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