Bubble-induced platelet aggregation in a rat model of decompression sickness

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Pontier JM, Vallée N, Bourdon L. Bubble-induced platelet aggregation in a rat model of decompression sickness. J Appl Physiol 107: 1825–1829, 2009. First published October 22, 2009; doi:10.1152/japplphysiol.91644.2008.—Previous studies have highlighted that bubble-induced platelet aggregation is a predictor index of decompression sickness (DCS) severity in animals and bubble formation after a single air dive in humans. The present study attempted to investigate plasmatic indexes of the coagulation system and platelet activation in our rat model of DCS. Male Sprague-Dawley rats were assigned to one experimental group with a hyperbaric exposure and one control group maintained at atmospheric pressure. Rats were compressed to 1,000 kPa (90 m saltwater) for 45 min while breathing air. The onset of death time and DCS symptoms were recorded during a 30-min observed period after rats had surfaced. Plasmatic indexes were platelet factor 4 (PF4) for platelet activation, soluble glycoprotein V (sGPV) for thrombin generation, and thrombin-antithrombin complexes for the coagulation system. Blood samples for a platelet count and markers were taken at 3 wk before the experimental protocol and within the 30 min after rats had surfaced. We confirmed a correlation between the percent fall in platelet count and DCS severity. Plasmatic levels of sGPV and PF4 were significantly increased after the hyperbaric exposure, with no change in the control group. The present study confirms platelet consumption as a potential index for evaluating decompression stress and DCS severity. The results point to the participation of thrombin generation in the coagulation cascade and platelet activation in bubble-induced platelet aggregation. In our animal model of DCS, the results cannot prejudge the mechanisms of platelet activation between bubble-induced vessel wall injury and bubble-blood component interactions.

As DCS is partly the consequence of the bubble-induced mechanical obstruction of vessels, blood platelets are likely to play a key role in the pathogenesis of the disease (22). Changes in the blood platelet count (PC) after hyperbaric exposure and decompression have been reported in an animal study (30) and in divers without clinical symptom (3, 9, 20, 26, 31, 37–39). In a previous study (33), we highlighted the relationship between the postdive decrease in PC and the magnitude of bubble formation in divers without DCS.

In DCS, experiments have strongly suggested a role for coagulation system activation as disseminated intravascular coagulation (15) and blood platelet aggregation in the pathogenesis of the disease. In a rat model of DCS, Philp et al. (28) demonstrated the central role of platelets in the formation of microthrombi in lung vessels after decompression. Moreover, adherence and aggregation of platelets to the bubble surface have been demonstrated in severe cases of DCS (29). In a previous study (32), we highlighted a relationship between the postdive decrease in PC and DCS severity in a rat model.

Several plasma markers can be used to characterize thrombosis occurring in the arterial network (coronary syndrome, ischemic stroke, etc.) or in veins (deep vein thrombosis, etc.) (2, 4, 5). Three markers have been studied particularly closely in the literature on this subject (1, 18, 34). Platelet factor 4 (PF4) is secreted by platelets, and its presence in the bloodstream can be correlated to the degree of platelet activation (34). The thrombin-antithrombin complex (TAT) and soluble glycoprotein V (sGPV) are both markers that reflect thrombin production (1). sGPV reflects the binding of platelets with the von Willebrand factor receptor when they adhere to the subendothelium during normal hemostasis. This marker has been studied in experimental models of acute thrombosis in rats and humans. Its detection is a recognized marker of thrombosis in atherosclerosis, atrial fibrillation, stroke, and myocardial infarction (34).

Few studies so far have examined the relationship between the occurrence of DCS and a thrombotic event resulting from the effects of bubbles on figured elements of the blood and vascular walls. Therefore, the objective of this study was to investigate platelet activation by measuring the PC and PF4, TAT, and sGPV levels in our experimental rat model of DCS (32).

MATERIALS AND METHODS

Study population. Fifty-seven male Sprague-Dawley rats (Charles River Laboratories) weighing 373 ± 18 g (mean ± SD) were used. Rats were kept at 22 ± 1°C under a 12:12-h light-dark cycle (lights on at 7:00 AM) with standard laboratory food and water available ad libitum. Before the experiments, rats were housed in an accredited animal care facility. Procedures were in accordance with the European Communities Council rules (Brussels, Belgium) directive of Novem-
and PF4 secretion and to minimize platelet activation, samples were
not drawn from an arterial puncture. Stroking the rat’s tail gently
resulted in blood droplets forming at the incision. Up to 20 μL of
blood were collected within 90 s by specially trained staff. Samples
were collected into an Eppendorf tube 3 wk before the experimental
protocol and within 30 min after the exposure in both groups. Samples
were anticoagulated by EDTA (12 mM) to avoid coagulation and
assayed for the PC with an Animal Blood Counter (Scil vet ABC, Scil,
France).

At the end of the protocol, animals were anesthetized with halothane
(5% with O2, Halothane Belamont) and killed by a lethal injection of pentobarbital (200 mg/kg ip, Sanofi Santé Animal).

Statistical analysis. Data are presented as means ± SD throughout.

RESULTS

In the experimental group (n = 49), death occurred inside
the hyperbaric chamber during the decompression phase or
immediately after the rats had surfaced in 29 rats. They were
excluded from the experimental protocol because of a too short
latency time to death for the blood sampling technique. Blood
samples for the PC and platelet activation markers were there-
fore performed in 20 rats (assayed rats).

In this group, blood PC values after hyperbaric exposure
significantly decreased compared with the preexposure values
(506 × 10^3 mm Hg^-3 ± 145 vs. 771 × 10^3 mm Hg^-3 ± 98, mean ±
SD, P < 0.001) with no significant change in the control group
(682 × 10^3 mm Hg^-3 ± 73 vs. 658 × 10^3 mm Hg^-3 ± 130, mean ±
SD, P = 0.74).

Pulmonary DCS symptoms with abnormal breathing, respira-
tory arrest, and, ultimately, death occurred in 7 of the 20
assayed rats [deceased DCS (D-DCS) group] within the 30-min
observation period. Neurological DCS symptoms, including
limb paralysis and walking difficulties, occurred in 11 of the 20
assayed rats [neurological DCS (N-DCS) group]. Finally, two
rats survived during the 30-min observation period after sur-
fac ing with no apparent DCS symptoms.

The percent fall in the PC was different in the D-DCS and
N-DCS groups (49.2 ± 16.0% vs. 28.6 ± 10.9%, respectively,
P < 0.01); it was only 20.4 ± 6.1% in the no DCS symptom
group, but the sample size was too small to consider this value
significant (Fig. 1).

No changes in the PF4, sGPV, and TAT plasma levels were
observed in the control group after the simulated hyperbaric
exposure (PF4: 1.3 ± 0.2 vs. 3.0 ± 0.8 ng/ml, P = 0.289;
sGPV: 0.7 ± 0.4 vs. 5.6 ± 1.9 ng/ml, P = 0.195; and TAT:
4.4 ± 1.7 vs. 11.0 ± 2.1 ng/ml, P = 0.23). Plasma levels of
platelet activation markers measured after the dive were sig-
nificantly increased (Fig. 2) compared with the predive values
in assayed rats for PF4 and sGPV (3.7 ± 0.8 vs. 9.9 ± 2.1
ng/ml for predive vs. postdive values of PF4, P = 0.014; and
4.5 ± 2.0 vs. 17.3 ± 4.5 ng/ml for predive vs. postdive values
of sGPV, P = 0.011). The increase observed for TAT was not
significant (10.4 ± 2.9 vs. 27.0 ± 8.1 ng/ml for predive vs.
postdive values, P = 0.053).
A negative correlation between PF4 activation marker values and the fall in the PC ($r^2 = 0.30$, $n = 19$, $P < 0.01$) was observed in the assayed rats; in this group, this relation was not observed between sGPV or TAT and the decreased PC.

**DISCUSSION**

We found that the blood PC measured immediately after the hyperbaric exposure was significantly decreased compared with the predive values; no change was observed in the control group. This result is in accordance with previous studies (30, 32) in different DCS rat models. Rats suffering from severe DCS with a short latency to death presented a more pronounced decline in the PC than surviving rats. Our results suggest a possible dose-response relationship between DCS severity and the magnitude of the PC decrease. A previous study (19) has demonstrated a relationship between DCS severity and death latency. This result confirms the findings of our previous study (32) in which we suggested that the postdive PC decrease could be a predictor of DCS severity after decompression in a rat model.

The results showed significantly increased plasma sGPV values in the experimental group after the hyperbaric exposure. This is an index of thrombin generation, which reflects the in vivo platelet activation and thrombotic status (34). In the bloodstream, circulating bubbles damage the vascular wall and activate endothelial cells. Mechanical damage to the vascular wall can go as far as a complete abrasion of the endothelium, revealing collagen and the subendothelial basal layer (25). Most tissues can be affected by these lesions. However, the pulmonary capillary network is the first to be locally affected by the formation of bubbles (7).

Physiologically, the purpose of platelet activation and aggregation is to immediately fill the vascular lesion by forming a clot of platelets. The interaction of platelets with the vascular wall plays a key role in normal hemostasis, ensuring vascular function and preventing hemorrhages in vessels in the microcirculation. Damage to the vascular wall causes the vessel to contract, triggers changes in local hemodynamic and rheological conditions, and, in particular, exposes the extracellular matrix of connective tissue. Although the resting endothelial cell presents a thromboresistant surface, the collagen-rich subendothelium can cause thrombosis. The effects of hemodynamic forces and von Willebrand factor cause the platelets to adhere to the subendothelium. This is the first stage of hemostasis. Alongside this platelet activation, the coagulation system is also activated. The damaged endothelial cells release tissue factor, which, in conjunction with factor VII, initiates the coagulation process and rapid thrombin formation. Activated platelets secrete the contents of their granules and initiate the arachidonic acid pathway. The ADP secreted and thromboxane A2 formed induces platelet aggregation via their receptors, the GPIIb/IIIa complex, if fibrinogen is present. This creates a platelet plug, which fills the blood vessel lesion. In certain diseases, however, these mechanisms can be activated in an uncoordinated way and can lead to thrombosis. In DCS, the presence of thrombin is more likely linked to the adhesion of platelets to the vessel’s damaged subendothelium. Thrombin, which is a powerful agonist, initiates platelet activation and thus the appearance of aggregates in the vascular system, which, in turn, leads to a thrombotic state, as is the case in disseminated intravascular coagulation (15, 28, 29).

In our experimental group, PF4 significantly increased after the hyperbaric exposure. PF4 is a specific platelet activation marker, and its presence in the plasma is a direct indicator of platelet activation. In vitro, platelet aggregation triggered by nitrogen bubbles causes blood platelets to decrease. The mechanisms of this aggregation are similar to those caused by platelet agonists such as ADP (37). Thorsen et al. (36) have suggested that bubbles are able to activate platelets in vitro by releasing ADP. Observations carried out in vitro using electron microscopes have shown that the induction of platelet aggregation by bubbles is linked to the contact and adhesion of plasma proteins and lipids to the interface between the liquid blood phase and the gaseous bubble phase (37). This process can be modified by pharmacological agents, in particular...
those that increase the intracellular cAMP concentration in platelets (36).

In vivo, Philp et al. (29) have shown that bubbles in the bloodstream would have the same effects as foreign bodies that come into contact with the figured elements of the blood, leading to platelet adhesion and aggregation around the bubbles. Geller (11) was the first to suggest the possibility of close interactions between bubbles in the bloodstream and platelets, although formal experimental proof was not provided. Others have demonstrated the presence of platelet aggregation around bubbles in the bloodstream during explosive decompression in rats (16) and the formation of platelet thrombi on histological preparations of dog pulmonary tissue after pathogenic decompression (8). Studies have demonstrated that the initial DCS event is linked to agglutination of the formed elements of blood during decompression, and the researchers suggested that the aggregations of platelets then behaved like emboli (10, 14, 17, 30). The interface between the gas and blood could bring about coagulation events, activating the complement system and fibrinolytic cascade (13, 27). At the blood bubble interface, platelets have been observed clustering or sticking to the bubble surface (12, 35).

In conclusion, if DCS is partly the consequence of the bubble-induced mechanical obstruction of vessels, then a thrombotic event could play a key role in the pathogenesis of the disease. The results point to the participation of thrombin generation, a powerful platelet agonist, and platelet activation in bubble-induced platelet aggregation. In our animal model of DCS, the results cannot prejudge between bubble-induced vessel wall injury and bubble-blood component interactions in platelet activation.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

REFERENCES


