

Mechanism of action of antiplatelet drugs on decompression sickness in rats: a protective effect of anti-GPIIb/IIIa therapy

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Lambrechts K, Pontier J-M, Mazur A, Theron M, Buzzacott P, Wang Q, Belhomme M, Guerrero F. Mechanism of action of antiplatelet drugs on decompression sickness in rats: a protective effect of anti-GPIIb/IIIa therapy. *J Appl Physiol* 118: 1234–1239, 2015. First published March 19, 2015; doi:10.1152/jappphysiol.00125.2015.—Literature highlights the involvement of disseminated thrombosis in the pathophysiology of decompression sickness (DCS). We examined the effect of several antithrombotic treatments targeting various pathways on DCS outcome: acetyl salicylate, prasugrel, abciximab, and enoxaparin. Rats were randomly assigned to six groups. *Groups 1* and *2* were a control nondiving group (C; $n = 10$) and a control diving group (CD; $n = 30$). Animals in *Groups 3* to *6* were treated before hyperbaric exposure (HBE) with either prasugrel ($n = 10$), acetyl salicylate ($n = 10$), enoxaparin ($n = 10$), or abciximab ($n = 10$). Blood samples were taken for platelet factor 4 (PF4), thiobarbituric acid reactive substances (TBARS), and von Willebrand factor analysis. Onset of DCS symptoms and death were recorded during a 60-min observation period after HBE. Although we observed fewer outcomes of DCS in all treated groups compared with the CD, statistical significance was reached in abciximab only (20% vs. 73%, respectively, $P = 0.007$). We also observed significantly higher levels of plasmatic PF4 in abciximab (8.14 ± 1.40 ng/ml; $P = 0.004$) and enoxaparin groups (8.01 ± 0.80 ng/ml; $P = 0.021$) compared with the C group (6.45 ± 1.90 ng/ml) but not CD group (8.14 ± 1.40 ng/ml). Plasmatic levels of TBARS were significantly higher in the CD group than the C group (49.04 ± 11.20 μ M vs. 34.44 ± 5.70 μ M, $P = 0.002$). This effect was prevented by all treatments. Our results suggest that abciximab pretreatment, a powerful glycoprotein IIb/IIIa receptor antagonist, has a strong protective effect on decompression risk by significantly improving DCS outcome. Besides its powerful inhibitory action on platelet aggregation, we suggest that abciximab could also act through its effects on vascular function, oxidative stress, and/or inflammation.

platelet activation; decompression illness; abciximab

A RAPID DECREASE IN AMBIENT PRESSURE, such as what occurs during self-contained underwater breathing apparatus (SCUBA) diving, may result in decompression sickness (DCS). DCS manifests in a broad array of symptoms that can result in severe morbidity, life-long disabilities, and even death (19). Postdecompression phenomena include physiopathological events such as vascular dysfunction (4), microcirculatory alterations (17, 18), inflammation process (7, 8), oxidative stress (9, 24, 25), and platelet activation (29), any of which could play a substantial role in the development of DCS. Among healthy humans, a positive correlation between bubble formation in-

tensity and magnitude of decrease in platelet count (PC) after diving has been previously shown (1, 31). In a rat model, the decrease in PC after diving correlated with severity of DCS (30, 32), indicating that platelet aggregation is associated with the pathophysiology of DCS (2, 5). Moreover, clopidogrel, an inhibitor of receptor P2Y₁₂ reducing ADP-induced platelet activation (11), was shown to both reduce DCS severity and decrease PC following decompression (33). Thus circulating bubbles are thought to stimulate the clotting system to a procoagulant state, indicating that platelet activation and aggregation are pivotal in the pathophysiology of DCS.

Moreover, an increase of plasmatic concentration of VCAM-1-associated endothelial microparticles after diving was reported (39), suggesting endothelial cell activation after SCUBA diving that may amplify platelet activation. Indeed, activated endothelial cells are known to inhibit anticoagulant mechanisms while stimulating procoagulant ones by releasing substances such as tumor necrosis factor, which stimulates the production of procoagulant tissue factor.

In addition, platelet function is modulated by reactive oxygen species (ROS) that are also routinely encountered after diving (9, 39). First, superoxide anions ($O_2^{\cdot-}$) in the blood quickly interact with NO to form peroxynitrite ($ONOO^-$) and decrease both NO availability and its antiplatelet effect (16). Second, hydrogen peroxide (H_2O_2) potentiates platelet aggregation in response to thrombin, collagen, and thromboxane A₂ (TXA₂), stimulates arachidonic acid liberation by phospholipase A₂, and increases intracellular Ca^{2+} , furthering platelet activation (10). One of the most immediate responses of platelets to a variety of activating stimuli is the production of the potent oxygen free radical, superoxide anion ($O_2^{\cdot-}$), thus increasing ROS production (20). Krotz et al. (14) convincingly suggest that collagen activation induces NAD(P)H oxidase-dependent $O_2^{\cdot-}$ release in platelets, which in turn enhances availability of released ADP, resulting in increased platelet recruitment.

Because of the involvement of platelets in DCS, the use of antiplatelet therapy and anticoagulants is relevant although their exact mechanism of action is still unknown, and randomized controlled studies concerning their efficacy remain absent. Platelet activation can occur from a variety of different stimuli and pathways, all mechanisms converging toward the glycoprotein (GP) IIb/IIIa complex (35) (Fig. 1). Acetylsalicylic acid (aspirin/ASA) inhibits platelet activation via acetylation of the cyclooxygenase enzyme (COX). The acetylation occurs rapidly and leads to irreversible inhibition of the COX enzyme. Low doses of ASA inhibit the activity of COX-I, decreasing the production of TXA₂ and platelet aggregation (28). However, in addition to this commonly used antiplatelet agent,

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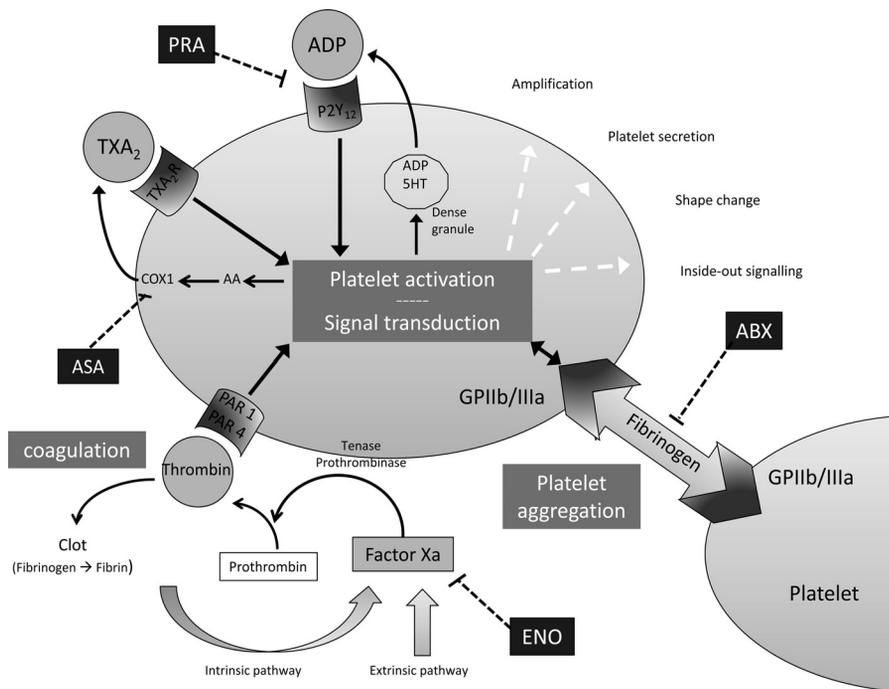


Fig. 1. Platelet activation mechanisms converging toward the glycoprotein (GP) IIb/IIIa complex. PRA, prasugrel; ASA, acetyl salicylic acid; ENO, enoxaparin; ABX, abciximab; TXA₂, thromboxane A₂; COX, cyclooxygenase enzyme; PAR, protease-activated receptor; AA, amino acid; 5-HT, serotonin.

distinct classes of antithrombotic agents with distinct mechanisms of action, GPIIb/IIIa antagonists (e.g., abciximab/ABX, eptifibatid) and antagonists of the ADP receptor P2Y₁₂ (e.g., prasugrel/PRA, clopidogrel), are used for the treatment and prevention of several cardiovascular pathologies (22). Besides this, because of multiple synergetic pathways of platelet activation and their close interplay with coagulation, current treatment strategies for treatment and prevention of cardiovascular problems are based on, not only platelet inhibition, but also the attenuation of procoagulant activity and the inhibition of thrombin generation (e.g., with enoxaparin/ENO) (13).

This study explored pharmacological interventions (ASA, PRA, ABX, and ENO), targeting different pathways of platelet inhibition, in a rat model of DCS. Examining whether any of these drugs might have a beneficial clinical effect on the incidence or severity of DCS, our goal was to distinguish the different platelet activation pathways and to clarify mechanisms behind procoagulant states as a consequence of diving. For this, we focused on coagulation through the inhibition of the factor Xa, platelet activation pathways (TXA₂ and ADP), and the aggregation process (through GPIIb/IIIa receptors). We also aimed to further elucidate possible mechanisms of pre-treatment with ASA, PRA, ABX, or ENO by measuring plasmatic markers of platelet activation (platelet factor 4, PF4), endothelial activation (von Willebrand factor, vWF), and oxidative stress (thiobarbituric acid reactive substances, TBARS). By correlating these markers with postdecompression DCS status, we aimed to identify possible relationships between oxidative stress, endothelial activation, and platelet activation in the pathophysiology of DCS.

MATERIALS AND METHODS

Study population. Ninety male Sprague Dawley rats aged 11 wk old at the day of the hyperbaric (HB) exposure were obtained from Janvier SAS (Le Genest-Saint Isle, France). The rats were housed in the university vivarium for at least 7 days after arrival, two per cage

under controlled temperature ($21 \pm 1^\circ\text{C}$) and lighting (12 h of light per day, 0800–2000). Because increased weight has been reported as a risk factor for DCS, we avoided a wide variation in the weight of the rats. Thus they were fed standard rat chow, which was adjusted individually. Water was supplied ad libitum. Animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication no. 85-23, revised 1996) and with the approval of the Université de Bretagne Occidentale animal research ethic committee (approval no. 01462). This study accords with recognized ethical standards and national/international laws and adheres to guiding principles for the care and use of vertebrate animals in research.

Study design. The rats were randomly and blindly assigned to one of the following six groups: *Group 1*, the control group (C; $n = 10$), did not receive any treatment and was not submitted to HB exposure. *Group 2*, the control diving group (CD; $n = 30$), did not receive any treatment but was submitted to HB protocols. *Groups 3 to 6* received one of the following four antithrombotic treatments before identical HB exposure: *Group 3*, the PRA group ($n = 10$); *Group 4*, the ASA group ($n = 10$); *Group 5*, the ENO group ($n = 10$); and *Group 6*, the ABX group ($n = 10$). For 1 h after atmospheric or HB exposure, the rats were observed for the appearance of signs of DCS (Fig. 2). After the observation period, the rats were anesthetized with pentobarbital (50 mg/kg) by intraperitoneal injection to collect blood samples for ELISA analysis. Following this, the rats were euthanized by a lethal intraperitoneal injection of pentobarbital.

Drug protocol. Methods of drug administration and doses were determined by a review of relevant literature. PRA (Eflent, Lilly, France) and ASA (Aspégic; Sanofi Aventis, Paris, France) were dissolved in water and administrated by oral gavage using a 1-ml syringe and a rodent-feeding needle. PRA was delivered 4 h before the experiment at a dose of 5 mg/kg (26, 37). ASA was administered once a day (100 mg/kg) for 2 days before the HB exposure (33). ENO (Lovenox, Sanofi Aventis) was injected subcutaneously (30 mg/kg) 2 h before the experiment (38). ABX (Reopro, Lilly, France) was injected intravenously (1 mg/kg) 30 min before HB exposure (15, 27).

HB protocol. The HB protocol was already described in our previous studies. Each rat was positioned in a 130-l steel HB chamber, always at the same hour to avoid interference with biological rhythm.

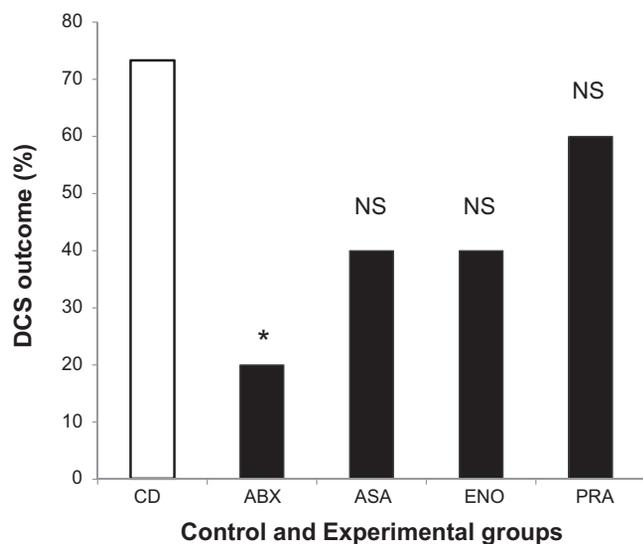


Fig. 2. Percentage of symptomatic rats suffering from decompression sickness (DCS) (lethal and nonlethal) within 60 min after surfacing. The group of control diving rats (CD, $n = 30$) is represented with the open bar; the different groups of treated rats, ABX ($n = 10$), ASA ($n = 10$), ENO ($n = 10$), and PRA ($n = 10$), are represented with the solid bars. Statistical comparisons are made between each treated group and the CD group. * $P < 0.05$ between groups. NS, nonsignificant ($P \geq 0.05$).

Air was used as a breathing mixture. Groups 2 to 6 were compressed with air at a rate of 100 kPa/min up to 1,000 kPa absolute pressure (90 meters of sea water, msw) and remained at maximum pressure for 45 min. Thereafter, decompression was performed at a rate of 100 kPa/min before pausing for three decompression stops: 5 min at 200 kPa (10 msw), 5 min at 160 kPa (6 msw), and 10 min at 130 kPa (3 msw). Total HB exposure duration for the protocol was 83 min. All dive depths were monitored using a modified personal dive computer (Puk; Mares, Rapallo, Italy). This protocol has been used in our previous experiments and reliably induces about 70% of DCS (21). The C group was similarly confined but not exposed to elevated ambient pressure and observed for 1 h before physiological investigation.

Classification of DCS. Animals were scored as having DCS only when one or more of the following symptoms were noted: respiratory distress, difficulty walking, paralysis convulsions, or death. The classification of DCS and analytical method were decided from a priori data before the experiment. We have classified their outcome variables as DCS vs. non-DCS.

Blood sampling and ELISA. Intracardiac blood collection was performed immediately following anesthesia or death in a BD Vacutainer citrate tube (0.11 M) and in 2-ml Eppendorf tubes with 30 μ l 7.5% EDTA as an anticoagulant. Blood was centrifuged at 1,000 g and 4°C for 10 min (samples for PF4 and TBARS analysis) and at 3,000 g , 4°C for 8 min (vWF samples). Collected plasma was aliquoted and stored at -80°C until the assay. PF4, vWF, and TBARS plasmatic concentrations were determined using commercially available ELISA kits for PF4 (Usen Life Science, Houston, TX), for vWF (Cusabio Biotechnology, Wuhan, China), and for TBARS (Cayman Chemical, Ann Arbor, MI). Assay procedures were performed according to each provider's instructions.

Statistical analysis. Data were stored in Microsoft Excel (Microsoft, Redmond, WA) and analyzed using SAS ver. 9.3 (SAS, Cary, NC). Data are expressed as means \pm SE, with n indicating the number of experiments performed.

Differences between the concentrations of plasmatic markers after diving simulation were first of all analyzed for normality. If the data were parametric, we proceeded with one-way ANOVA. Upon iden-

Table 1. DCS-specific outcomes in different groups

	CD Group	ABX Group	ASA Group	ENO Group	PRA Group
Lethal DCS	60%	10%	40%	30%	40%
Nonlethal DCS	13%	10%	0%	10%	20%
Total DCS	73%	20%	40%	40%	60%
Non-DCS	26.70%	80%	60%	60%	40%

Groups included lethal decompression sickness (DCS) (symptoms inducing death within 60 min following diving), nonlethal DCS (DCS symptoms without death), total number of DCS, and non-DCS (animals without any symptoms). CD, control diving group; ABX, abciximab; ASA, acetyl salicylic acid; ENO, enoxaparin; PRA, prasugrel.

tifying significant differences in the ANOVA, a Dunnett's post hoc test was used to investigate the relevant parameters. For blood marker analysis, upon identifying significant differences in the ANOVA, a Dunnett's post hoc test was used to investigate the relevant parameters. For the nonparametrically distributed results, we ran a Kruskal-Wallis test. Significance was accepted at $P \leq 0.05$. Results are expressed as means \pm SD; n indicates the number of rats.

RESULTS

Clinical observations. Rats with observable signs of DCS died within 30 min after HB exposure, most often in the few minutes following decompression. Although the proportion of DCS was less after all treatment compared with CD group, statistical significance was reached in the ABX group only (20% vs. 73%, respectively, $P = 0.011$). There was no significant difference in DCS outcome among the control group and PRA, ASA, and ENO groups. DCS occurred in 40% of rats in the ASA group, 40% of rats in the ENO group, and in 50% of rats in the PRA group (Fig. 1) (Table 1). There were no observable DCS cases or deaths among the C group.

Blood marker analysis. The results highlighted a significantly higher mean level of plasmatic PF4 for CD group compared with the C group (8.14 ± 1.40 ng/ml vs. 6.45 ± 1.90 ng/ml; $P = 0.016$) after HBE (Fig. 3). Similarly, an upper level of plasmatic PF4 was found in the ENO group compared with controls (8.01 ± 0.80 ng/ml vs. 6.45 ± 1.90 ng/ml; $P = 0.009$). ANOVA test showed no significant change in mean

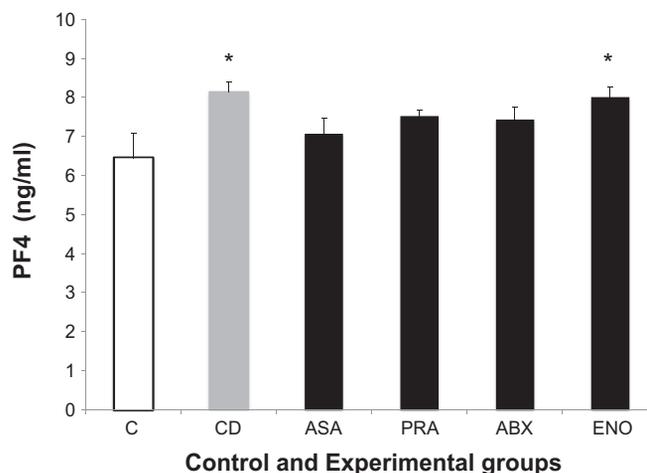


Fig. 3. Platelet factor 4 (PF4) (ng/ml) concentrations after decompression. C, control nondiving group, open bar ($n = 10$). CD group, shaded bar ($n = 30$). Treated groups (solid bars): ASA ($n = 10$), PRA ($n = 10$), ABX ($n = 10$), and ENO ($n = 10$). * $P < 0.05$ compared with C group. Data are means \pm SE.

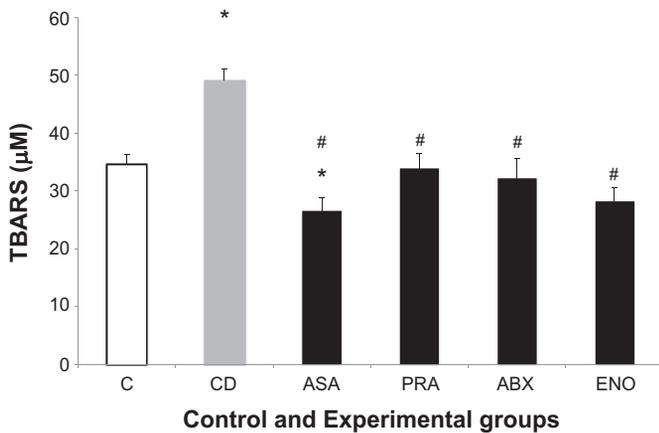


Fig. 4. Thiobarbituric acid reactive substances (TBARS) (μM) concentrations after decompression: C group, open bar ($n = 10$). CD, shaded bar ($n = 30$). Treated groups (solid bars): ASA ($n = 10$), PRA ($n = 10$), ABX ($n = 10$), and ENO ($n = 10$). * $P < 0.05$ compared with C group; # $P < 0.05$ compared with CD group. Data are means \pm SE.

plasmatic PF4 concentration in ABX (7.43 ± 1.10 ng/ml), ASA (7.06 ± 1.30 ng/ml), and PRA groups (7.51 ± 0.50 ng/ml) compared with the C group. Although the treatments in this experiment could potentially prevent PF4 increase after diving, we did not observe any difference between treated groups and the CD group (ASA: $P = 0.139$; PRA: $P = 0.625$; ABX: $P = 0.269$; ENO: $P = 0.305$) (Fig. 3).

We found a significantly higher plasmatic level of TBARS in CD group after HB exposure compared with C group controls (49.04 ± 11.20 μM vs. 34.44 ± 5.70 μM , $P = 0.013$). A lower level of TBARS was measured in the ASA group compared with the C group (26.41 ± 7.90 μM vs. 34.44 ± 5.70 μM , $P = 0.027$). No difference was observed between C group and the other treated groups (Fig. 4): ABX (32.01 ± 11.40 μM), ENO (28.08 ± 7.90 μM), or PRA (33.7 ± 8.8 μM). Compared with the CD group, TBARS concentrations remained significantly lower in the treated groups (Fig. 4): ASA ($P = 0.0002$), PRA ($P = 0.007$), ABX ($P = 0.001$), and ENO ($P = 0.0001$).

None of the groups showed a significant variation in vWF plasmatic levels (CD: 3.07 ± 0.70 $\mu\text{g/ml}$; ABX: 2.87 ± 0.40 $\mu\text{g/ml}$; ASA: 3.5 ± 1.1 $\mu\text{g/ml}$; ENO: 2.81 ± 0.50 $\mu\text{g/ml}$; PRA: 2.44 ± 1.40 $\mu\text{g/ml}$) following decompression, compared with C group (2.64 ± 0.60 $\mu\text{g/ml}$), as shown in Fig. 5.

DISCUSSION

Since recognizing DCS as a series of pathological responses in cascade, including vascular dysfunction, inflammation, oxidative stress, and platelet activation/aggregation, rather than a phenomenon exclusive to gas supersaturation and bubble load, we feel that it appears relevant to pursue antithrombotic therapies to treat DCS symptoms. By using antiplatelet and anticoagulant drugs targeting different steps of the coagulation cascade, we aimed to clarify the mechanisms of diving-induced platelet aggregation. In this study, we focused on the link between DCS and coagulation through the inhibition of the factor Xa, platelet activation pathways (TXA₂ and ADP), and the aggregation process (through GPIIb/IIIa receptors).

Our rat model of decompression showed a DCS incidence of 73% in the DC group. Consistent with available data, we also

measured significantly higher PF4 and TBARS levels. Otherwise, the exposure to our HB protocol did not influence vWF levels, confirming our previous results that found no endothelial activation after diving (18).

The main finding in this study is that rats treated with ABX had a significantly lower DCS incidence than nontreated rats after HB exposure. This finding strongly supports the hypothesis that platelet aggregation plays a major role in the occurrence of DCS observed after a single dive.

To determine whether coagulation could participate in DCS, pretreatment with ENO has been used. ENO acts primarily on the coagulation factor Xa but also, to a lesser degree, on thrombin. Centered at the convergence of the intrinsic and extrinsic coagulation pathways, factor Xa transforms prothrombin into thrombin. Thrombin is crucial for the formation of fibrin, an essential component of clots. Antithrombin activity of ENO limits amplification of the coagulation cascade. Our results using ENO pretreatment suggest no significant beneficial effect of reducing the coagulation system to decrease DCS occurrence. These results confirm those of Pontier et al. (33), who find no convincing reason to use anticoagulant therapy following the administration of heparin to rats before a simulated dive. They also indicate that mechanisms occurring before the coagulation itself are involved in the development of DCS.

The implication of platelet activation in DCS was first evaluated through the use of ASA, an irreversible inhibitor of the COX-I pathway. Although ASA is the most widely used antiplatelet agent prescribed as an adjunctive measure in the treatment of DCS in France, our results confirm those of previous studies (3, 23, 33). A decrease in DCS outcome after ASA pretreatment failed to meet statistical significance, despite a positive effect on PF4 and TBARS levels compared with the CD group. These results differ from those of Popovic et al. (34), who showed a significant decrease in DCS incidence following ASA administration. In this study, ASA was administered daily during a longer period of 1 mo before the simulated dive, whereas our dose was administered more acutely, over just 2 days before compression. These differences may have contributed to the disparate results, and this in itself

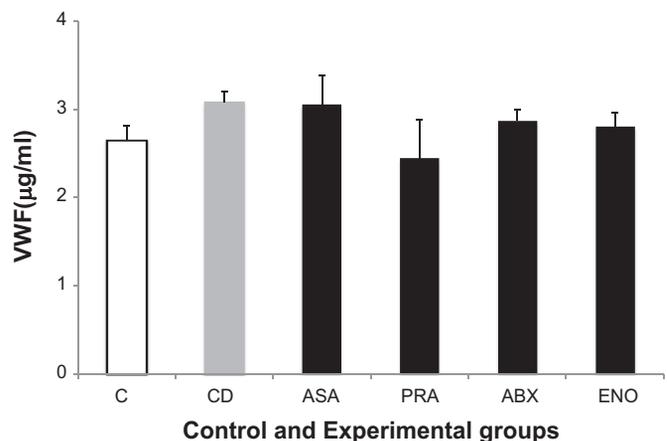


Fig. 5. von Willebrand factor (vWF) ($\mu\text{g/ml}$) TBARS (μM) concentrations after decompression: C group, open bar ($n = 10$). CD group, shaded bar ($n = 30$). Treated groups (solid bars): ASA ($n = 10$), PRA ($n = 10$), ABX ($n = 10$), and ENO ($n = 10$). * $P < 0.05$ compared with C group; # $P < 0.05$ compared with CD group. Data are means \pm SE.

may yet prove interesting, given that adjunctive treatment with ASA for DCS is necessarily acute (upon presentation for recompression). Our data show that ASA prevented an increase of PF4 and significantly reduced TBARS production. It is well documented that ASA reduces production of ROS via its propensity to scavenge superoxide anions ($O_2^{\cdot-}$) by reducing NADPH activity (6, 36). This may explain why we found lower levels of TBARS in the ASA group compared with controls. However, even if ASA was shown to be efficient at preventing platelet activation by reducing PF4 release in response to dense granules of activated platelets, we nonetheless conclude that the COX pathway does not appear likely as the predominant pathway leading to DCS, given that DCS outcome was not altered significantly.

The implication of platelet activation in DCS has also been analyzed through the purinergic receptor pathway. Similar to ASA, PRA pretreatment was effective at preventing platelet activation (PF4) and an oxidative stress increase (TBARS) compared with the CD group. However, there was only a trend toward lower DCS incidence following this treatment. PRA was chosen for its selective and irreversible effect on P2Y₁₂ receptors on platelets, resulting in noncompetitive inhibition of ADP-induced platelet fibrinogen binding and finally platelet aggregation and reduced stability of platelet aggregates (11). A previous study showed promising results with the use of clopidogrel (another ADP receptor antagonist) pretreatment protective against DCS in rats (33). The antiaggregant potency of PRA has been shown to be at least 10 times higher and faster than clopidogrel in both rats and humans because of the more efficient generation of its active metabolite (trifluoroacetic acid salt) (37). Thus we were expecting a similar or even better effect of PRA to inhibit decompression-induced platelet aggregation. From our results, platelet activation through the ADP pathway does not appear to play a primary role in the pathophysiology of the disease. We assume that such a divergence between the effects of clopidogrel and PRA on DCS outcome could be attributable to the positive additional effects of clopidogrel administration on vascular injury and/or inflammation status. In previous research, we have shown a reduction in endothelium-dependent and endothelium-independent macrovascular and microvascular function after diving (17, 18). Vascular dysfunction is thought to play a deleterious role in the pathophysiology of DCS. A study from Heitzer et al. (12) showed that clopidogrel improved endothelial nitric oxide bioavailability and diminished inflammation in patients with symptomatic coronary artery disease. These elements, taken together with the low effect of PRA, suggest that, beyond the inhibition of platelet aggregation, the positive effect of clopidogrel could be attributable to its vasoprotective effects.

ABX was chosen for its powerful and rapid inhibition of platelet function by blocking the final common pathway of platelet activation, the aggregation. Our results showed that inhibition of this final step with ABX seemed to be much more efficient than independently blocking a single pathway with aspirin or PRA. We suggest that the more powerful action on platelets by blocking all activation pathways was efficient enough to decrease DCS outcome significantly, whereas the blockade of a single pathway with clopidogrel or PRA could have been counteracted by other activation pathways, thus showing no effect on DCS. However, an alternative hypothesis is that, similar to clopidogrel, ABX may have counteracted the

physiopathological cascade leading to DCS in the present study also by triggering microvascular alteration and vascular dysfunction. GPIIb/IIIa receptor antagonists like ABX or eptifibatide have been shown to reduce endothelial leakage and to improve microcirculatory disturbances during an experimental model of endotoxemia (40, 41). The protective properties of ABX on microcirculation and endothelial leakage seem to be leukocyte independent, and this indicates a possible role of platelets because platelet-activating factor and serotonin-receptor antagonist are also known to be protective (42, 43). GPIIb/IIIa inhibition with ABX results in a significant reduction of macromolecular efflux during leukocyte-dependent endotoxemia. Both pre- and posttreatment with ABX reduces macromolecular leakage during leukocyte-dependent endotoxemia and prevents an increase in leukocyte adherence. GPIIb/IIIa receptor antagonists have also been found as protective by reducing vasodilatory response to acetylcholine, induced by apoptotic smooth muscle cell microparticles. Taking this together, we hypothesize that the beneficial effect of ABX against DCS could be attributable to the combined effects of a powerful antiplatelet action, protective effects on microcirculation, and reduced microparticle generation.

In conclusion, our results suggest that ABX pretreatment, a powerful GPIIb/IIIa receptor antagonist, has a strong protective effect on decompression risk by significantly improving the DCS outcome and reducing DCS severity. One explanation for ABX efficacy could be dose related. It could be that the administration dose chosen was more efficient than the doses chosen for the other treatments. This should be considered a limitation of the study. From a mechanistic point of view, we presume that the beneficial effect of ABX is attributable to its powerful antiplatelet action but also involves other combined and positive side effects on vascular function, oxidative stress, and inflammation. In a therapeutic approach, these findings may lead to a better understanding of the use of antiplatelet drugs in the medical treatment of DCS in humans. Further studies should be aimed at demonstrating whether ABX can minimize secondary pathophysiological pathways such as vascular dysfunction and might offer humans some benefit as an adjuvant in the treatment of DCS.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: K.L., J.-M.P., A.M., M.T., M.B., and F.G. conception and design of research; K.L., J.-M.P., A.M., M.T., P.B., Q.W., M.B., and F.G. performed experiments; K.L., P.B., and F.G. analyzed data; K.L., J.-M.P., M.T., and F.G. interpreted results of experiments; K.L. prepared figures; K.L., M.T., Q.W., and F.G. drafted manuscript; K.L., J.-M.P., A.M., P.B., Q.W., M.B., and F.G. edited and revised manuscript; K.L., J.-M.P., A.M., M.T., P.B., M.B., and F.G. approved final version of manuscript.

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