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Venous air embolism induces both platelet dysfunction and thrombocytopenia

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Background: *In vitro*, air bubbles can induce platelet activation and platelet to air bubble binding. We therefore tested *in vivo* the hypothesis that venous air embolism (VAE) induces (1) platelet dysfunction and (2) thrombocytopenia.
Methods: Adult swine (60.8 ± 3.9 kg; $n = 8$) were anaesthetized, mechanically ventilated, and placed in a semi-upright position. Air boli (0.5–80 ml) were injected randomly via an ear vein, and arterial blood was sampled after cumulative air dosages of 0, 80, 160, and 240 ml. Coagulation was assessed by impedance aggregometry, rotational thrombelastometry, whole blood count, plasmatic coagulation variables, and fibrinogen, D-dimer, protein C, and antithrombin plasma concentrations, respectively.
Results: VAE induced a 47% decrease in platelet count (303 vs. 160 nl^{-1}; $P < 0.001$) over the dose range assessed, with haematocrit being unaltered. Furthermore, VAE-impaired platelet aggregation induced by adenosine diphosphate, arachidonic acid, collagen, and the thromboxan analogue U46619 over the dose range assessed independent of thrombocytopenia. ($P < 0.05$ vs. baseline). In contrast, rotational thrombelastometry alone was quite insensitive in detecting VAE-induced coagulation changes, showing only at near lethal air dosages a prolonged clot formation time following activation with tissue factor, contact activator, and during spontaneous coagulation ($P < 0.05$ vs. baseline).
Conclusions: VAE induces both a dose-dependent decrease in platelet count and a marked decrease in platelet aggregation, independent of thrombocytopenia ($P < 0.05$ vs. baseline).

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VENOUS air embolism (VAE) is a frequent and potentially fatal complication during neurological surgery in the sitting position and may occur whenever venous pressure at the site of surgery is near or below atmospheric pressure.[1–3]

Intrapulmonary air following VAE is known to cause damage to the endothelium,[4,5] creating gaps between endothelial cells and facilitating pulmonary oedema.[6] Furthermore, air or VAE induces complement[7,8] and leads to platelet activation.[9,10] *In vitro*, direct platelet to air bubble binding has been described.[9,11] However, to our knowledge, there have been no studies addressing whether these effects are of importance for platelet function *in vivo* and to what extent blood coagulation is impaired by VAE. Obviously, impaired platelet function may increase the risk of intraoperative or post-operative bleeding, with potential disastrous sequelae in brain surgery.

Accordingly, we tested the hypothesis that VAE induces (1) platelet dysfunction and (2) thrombocytopenia.

Methods

The experimental protocol was approved by the local animal care committee (TSG-No.: G851/05), and animals were treated in accordance with the guidelines of the American Physiological Society and the Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, revised 1996).

Animal preparation

Eight male pigs [weight: 60.8 ± 3.9 kg standard deviation (SD)] were studied. After an overnight

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fast, the animals were pre-anaesthetized with ketamine [27 mg/kg intramuscularly (i.m.)], azaperone (2.2 mg/kg i.m.), and atropine (2 mg i.m.). After placement of an 18-G peripheral cannula into a left ear vein and injection of propofol (2 mg/kg), the animals were endotracheally intubated and mechanically ventilated (rate: 12 min^{-1}, tidal volume: 8 ml/kg, inspiratory oxygen fraction: 0.5). Anaesthesia was maintained by propofol (7 mg/kg/h) and fentanyl (5 μg/kg/h) intravenously.

For continuous arterial pressure monitoring and blood sampling, an 18-G catheter (Vygon GmbH, Aachen, Germany) was inserted into the right carotid artery, and an 8.5 F sheath (Arrow International Inc., Reading, PA) was inserted into the right jugular vein for placement of a 7.5 F pulmonary artery catheter (CritiCath. SP5507 TD Catheter, Becton Dickinson Inc., Sandy, UT). Additionally, via an 11 F sheath (Arrow International Inc.) in the right jugular vein, a 10 F intracardiac echocardiography (ICE) catheter (AcuNav, Siemens Medical Solutions, Erlangen, Germany) was placed into the superior vena cava for detection of VAE.

Measurements

Platelet function. Platelet function was analysed using impedance aggregometry (Multiplate, Dynabite Informationssysteme GmbH, Munich, Germany). [12] Platelets were activated by adding either 20 μl adenosine diphosphate (4 mM), arachidonic acid (30 mM), collagen (4 μg), or the thromboxane analogue U46619 (31 μM) to hirudin anticoagulated blood (25 μg/ml), as described previously.[12,13] Aggregation was expressed as area under the curve (AUC) of the aggregation time relationship for the first 7 min, as this best reflects platelet aggregation.[13,14]

Whole blood coagulation. Whole blood coagulation was measured using rotational thrombelastometry (ROTEM®, Pentapharm, Munich, Germany),[15–17] based on the traditional thrombelastogram as described by Hartert.[18] Citrated blood was used for all experiments and coagulation was assessed following recalcification using 0.2 M $CaCl_2$ (20 μl Star-TEM™, Pentapharm, Munich, Germany). Platelet activation was initiated with either 20 μl tissue thromboplastin (i.e., tissue factor and phospholipids, ExTEM™, Pentapharm) or partial thromboplastin (i.e., phospholipids and elagic acid, InTEM™, Pentapharm), respectively. Furthermore, coagulation after the addition of 20 μl Cytochalasin D (FibTEM™, Pentapharm), and spontaneous coagulation (NaTEM™, Pentapharm) were assessed. Whole blood count, plasmatic coagulation variables (activated partial thromboplastin time, international normalized ratio, and thrombin time), and fibrinogen, D-dimer, protein C, and antithrombin plasma concentrations were also measured.

Experimental protocol

After instrumentation, animals were placed in a 45° semi-upright position[19] and eight increments of air (0.5, 1, 2, 5, 10, 20, 40, and 80 ml) were injected via an ear vein in a randomized order, with every air volume being injected twice. Different amounts of air were used to simulate the clinical setting of VAE, with different volumes of air often embolizing over time. Following each injection, a consecutive air injection was only performed when no more intracardiac air could be visualized by ICE, as described previously.[20] Whenever cardiocirculatory collapse occurred the experiment was terminated. The cumulative amount of air injected before the last injection inducing cardiocirculatory collapse was defined as 'near-lethal air dosage'.

Arterial blood for analysis of variables was drawn before air injections and after cumulative air dosages of 80, 160, and 240 ml, respectively.

Statistics

Data are presented as means ± standard deviation (SD), unless indicated otherwise. Statistical analysis was performed using the SPSS 13.0 Software package (SPSS Inc., Chicago, IL) and the Microsoft Excel software package (Microsoft Office XP, Microsoft, Redmond, WA).

Statistical differences in values between baseline and those following VAE were analysed by repeated measures ANOVA for platelet function analysis (four measurements), and the Wilcoxon's signed rank test for analysing whole blood coagulation (two measurements), respectively. Platelet aggregation values were also normalized to platelet count (AUC/ platelet count), so as to eliminate the effects of thrombocytopenia. An α-error $P<0.05$ was used to indicate statistical significance.

Results

Platelet count

VAE decreased the platelet count by 47% (303 vs. 160 nl^{-1}; $P<0.001$) over the dose range assessed

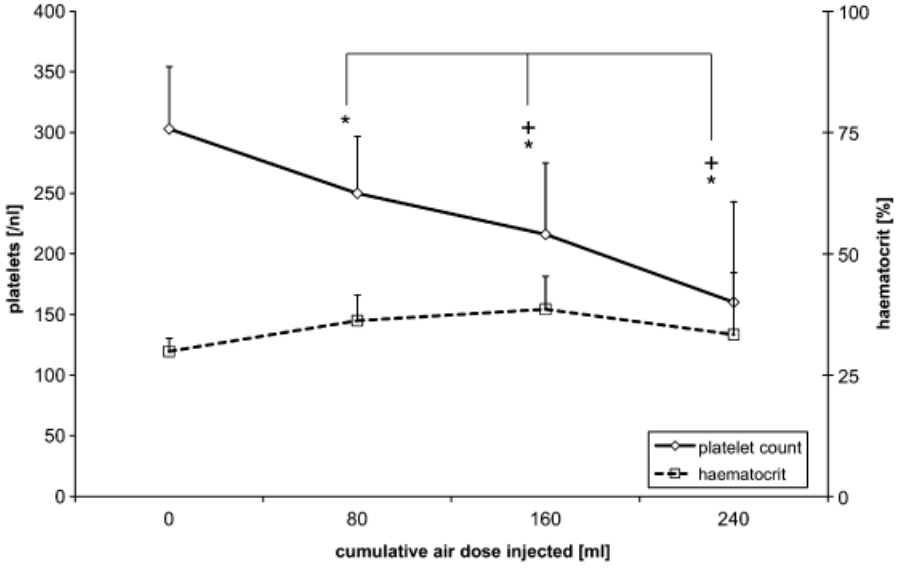

Fig. 1. Changes in platelet count following venous air embolism (VAE). Data (means ± SD) from swine subjected to VAE. VAE induced a dose-dependent decrease in the platelet count, with the haematocrit being unaltered. *P < *0.05 for platelet count vs. initial value.* $^{+}$P < *0.05 for dose-dependent effect in changes of the platelet count. SD, standard deviation.*

(0–240 ml of air injected), with haematocrit being unaltered (Fig. 1). The average amount of air inducing cardiocirculatory collapse was 4.6 ± 1.6 ml/kg.

Platelet function

VAE impaired platelet aggregation independent of thrombocytopenia and over the dose range assessed ($P < 0.05$ for all activations vs. baseline) regardless of whether induced by adenosine diphosphate, arachidonic acid, collagen, or the thromboxan analogue U46619. A cumulative air dose as small as 80 ml was already associated with a decrease in platelet aggregation ($P < 0.05$) when induced by ADP (− 26%), collagen (− 24%), or U46619 (− 64%). In contrast, 160 ml of air was required for a decrease (− 59%) in platelet aggregation observed after stimulation with arachidonic acid ($P < 0.05$) (Fig. 2).

Whole blood coagulation

Rotational thrombelastometry was less sensitive than impedance aggregometry in detecting VAE-induced coagulation changes, revealing diminished maximum clot firmness after a cumulative air dosage of 240 ml following activation with tissue factor (ExTEM™), elagic acid (InTEM™), and Cytochalasin D (FibTEM™). (Fig. 4) Following spontaneous coagulation, the decrease in maximum clot firmness observed almost reached statistical significance ($P = 0.058$). Clot formation time was only prolonged at near lethal air dosages

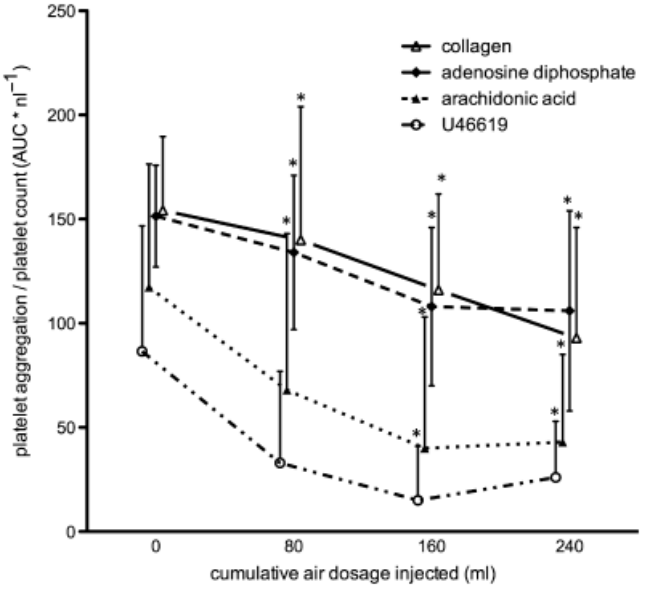

Fig. 2. Changes in platelet aggregation following venous air embolism (VAE). Data (means ± SD) from swine subjected to VAE. VAE reduced platelet aggregation as assessed by impedance aggregometry following stimulation by adenosine diphosphate, arachidonic acid, collagen, and the thromboxane analogue U46619. Data normalized to platelet count at the time of measurement. *P < *0.05 for platelet aggregation/platelet count vs. the initial value. SD, standard deviation.*

($P < 0.05$) following activation with tissue factor (ExTEM™) or elagic acid (InTEM™) (Fig. 3).

Plasmatic coagulation variables

Thrombin time, activated partial thromboplasmin time, and international normalized ratio were unaltered by VAE over the dose range assessed.

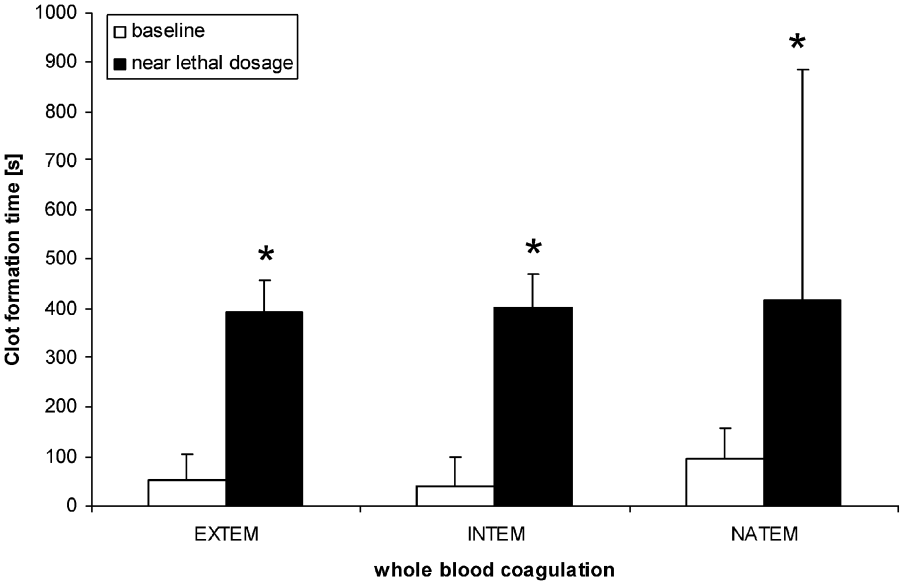

*Fig. 3. Changes in clot formation time (CFT) following venous air embolism (VAE). Data (means ± SD) from swine subjected to VAE. VAE induced a significant prolongation in CFT at near-lethal dosages when compared with the initial values. ExTEM™, platelet activation with tissue thromboplastin; InTEM™, platelet activation with partial thromboplastin; NaTEM™, spontaneous coagulation, no specific platelet activation. *P < 0.05 vs. initial value.*

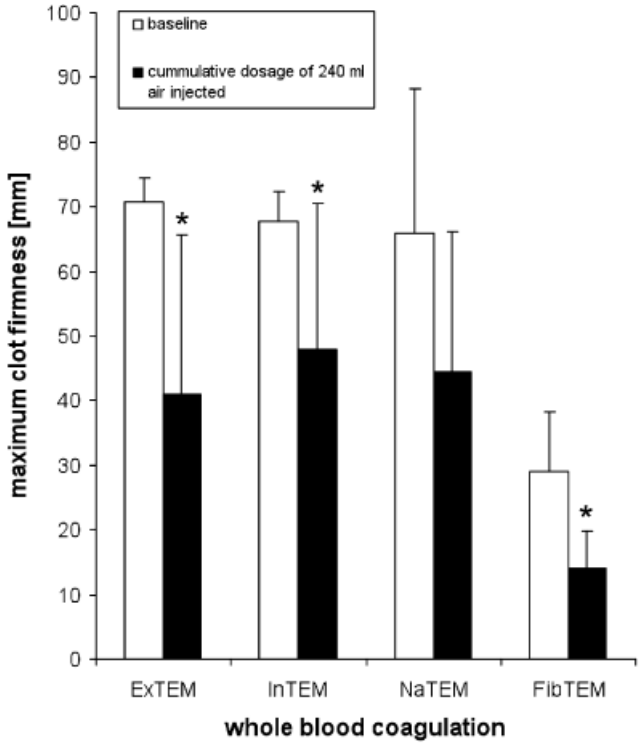

*Fig. 4. Changes in maximum clot firmness (MCF) following venous air embolism (VAE). Data (means ± SD) from swine subjected to VAE. VAE induced a significant decrease in MCF after a cumulative dosage of 240 ml of air injected when compared with the initial values. ExTEM™, platelet activation with tissue thromboplastin; InTEM™, platelet activation with partial thromboplastin; FibTEM™, platelet depletion with Cytochalasin D; NaTEM™, spontaneous coagulation, no specific platelet activation. *P < 0.05 vs. initial value.*

Furthermore, fibrinogen, D-dimer, protein C, and antithrombin III plasma concentrations remained unchanged.

Discussion

This study is the first to show that VAE induces both a dose-dependent decrease in platelet count and a marked decrease in platelet aggregation, independent of thrombocytopenia.

Microbubbles are known to affect blood clotting *in vitro* due to induction of coagulation and direct platelet aggregation.[10] Activated platelets adhere to air and coat air bubbles,[9,11] leading to stabilized air bubbles *in vitro*. These effects could *in vivo* prolong air reabsorption, aggravate haemodynamic effects, and cause marked thrombocytopenia.

The almost 50% decrease in the platelet count observed over the air dose range assessed could be attributable to two distinct pathophysiological effects. First, direct platelet to air bubble binding might have caused the decrease in the circulating platelet count because such binding has been shown *in vitro*.[11] Second, pulmonary air embolism releases mediators like endothelin, serotonin, or thromboxane, and activates the complement system, which in turn may induce platelet aggregation or pooling in the lung. The decline in circulating platelets observed following VAE is surprisingly large, considering that platelets are stored in various organs and platelet release could mask platelet consumption or pooling.

Furthermore, platelet aggregation, as assessed by impedance aggregometry, decreased following VAE irrespective of whether stimulation was induced by ADP, arachidonic acid, collagen, or U6619, and this effect was independent of the platelet count.

While we cannot pinpoint the mechanisms responsible for decreased aggregation following VAE

despite using a variety of different stimulants, some speculation about the pathophysiological pathways involved can be made. First, platelets are known to show differences in aggregability following stimulation with the same agent,[21–23] and, therefore, it is likely that the ones with the highest activatibility are the first to aggregate following VAE *in vivo*. Thus, platelet aggregation as measured by impedance aggregometry *in vitro* may have represented a fraction of platelets of lesser activatibility because a lesser number of platelets with a low threshold for activation may have circulated following VAE.

Second, following VAE, maximum clot firmness was also diminished following addition of Cytochalasin D (FibTEM™) for abolition of platelet function *in vitro*, revealing a decreased clot strength independent of the platelet function, despite plasmatic fibrinogen concentrations being unaltered over the dose range assessed. These data suggest that VAE may induce changes in fibrin polymerization, and additional studies are required to delineate further the effects of VAE on fibrin polymerization.

Thus, while plasmatic coagulation variables were unchanged over the entire VAE dose range assessed, maximum clot firmness decreased and clot formation time was prolonged as assessed by rotational thrombelastometry at near-lethal air dosages. Our data show, therefore, that impedance aggregometry in particular but also rotational thrombelastometry are sensitive tools to assess VAE-induced coagulation changes. Furthermore, VAE-induced coagulopathy is not mirrored in standard plasmatic coagulation tests.

Additionally, to the extent that our data can be extrapolated to humans, VAE may induce substantial thrombocytopenia and platelet dysfunction, and this could increase the risk for bleeding, in particular in patients with pre-operative platelet dysfunction or thrombocytopenia.

Our study has limitations. First, data were generated using swine, but it is impossible to perform such studies in humans. Second, our study was not designed to distinguish the effects exerted by direct effects of air on platelets from those induced by mediators and, therefore, further studies are needed to evaluate the pathophysiological pathways of VAE-induced platelet dysfunction.

In conclusion, VAE induces thrombocytopenia and a decrease in platelet aggregation independent of thrombocytopenia.

Acknowledgement

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