Thrombosis Research (2006) **118**, 341–352

ELSEVIER

THROMBOSIS RESEARCH

intl.elsevierhealth.com/journals/thre

REGULAR ARTICLE

Evaluation of a porcine model to study in vivo platelet activation

Eirik Søfteland [a,*], Tore Framstad [c], Astrid Nordvik [b], Anne M. Bakken [d], Chandru Punwani [e], Inge Morild [f], Tor Hervig [a], Terje Thorsen [a], Holm Holmsen [a]

[a] *Department of Biomedicine, University of Bergen, N-5021 Bergen, Norway*
[b] *Surgical Research Laboratory/Vivarium, University of Bergen, N-5021 Bergen, Norway*
[c] *Gilde Hed-Opp, Postboks 70, N-2360 Rudshøgda, Norway*
[d] *Laboratory of Clinical Biochemistry, Haukeland University Hospital, N-5021 Bergen, Norway*
[e] *Department of Heart Diseases, Haukeland University Hospital, N-5021 Bergen, Norway*
[f] *Department of Forensics, Haukeland University Hospital, N-5021 Bergen, Norway*

Received 14 June 2005; received in revised form 1 August 2005; accepted 2 August 2005
Available online 6 September 2005

KEYWORDS
Swine;
Adenosine diphosphate;
Platelet activating factor;
Scintigraphy;
Microscopy;
Platelets

Abstract

Introduction: In order to investigate if decompression sickness involves platelet activation an animal model was evaluated.
Materials and methods: Twenty-four thiopentone–midazolam–fentanyl-anaesthetized pigs in four groups received 5-min infusions of adenosine diphosphate (25 mg/kg) or platelet activating factor (0.4 μg/kg). Groups 1 and 2 (adenosine diphosphate, $n=6$ and platelet activating factor, $n=6$) were studied for 30 min and then sacrificed. Groups 3 and 4 (adenosine diphosphate, $n=6$ and platelet activating factor, $n=6$) were sacrificed immediately afterwards to study short-term changes. Haemodynamics, platelet counts and post mortem lung platelet aggregates were registered. Groups 1 and 2 also had indium platelet labelling, lung scintigraphy and platelet accumulation index calculations performed.
Results: Adenosine diphosphate induced immediate and more profound transient shocks. Platelet and leukocyte count decreases and occurrences of post mortem lung

Abbreviations: ACD, acidic citric dextrose; ADP, adenosine diphosphate; DAP, diastolic arterial pressure; DCS, decompression sickness; DPAP, diastolic pulmonary arterial pressure; IBP, invasive blood pressure; Iv., intravenously; ^{111}In, ^{111}Indium; ^{131}I, ^{131}Iodine; HR, heart rate; HSA, human serum albumin; LPS, lipopolysaccharide; PAF, platelet activating factor; PAI, platelet accumulation index; PA, pulmonary artery; PPP, platelet-poor plasma; PRP, platelet-rich plasma; ROI, region of interest; SAP, systolic arterial pressure; SPAP, systolic pulmonary arterial pressure.

* Corresponding author. Tel.: +47 55976850; fax: +47 55976898.
E-mail addresses: eirik.softeland@biomed.uib.no (E. Søfteland), tore.framstad@gilde.no (T. Framstad).

0049-3848/$ - see front matter
doi:10.1016/j.thromres.2005.08.003

platelet aggregates were significantly more profound in the 5-min adenosine diphosphate group (Group 3) than in the platelet activating factor group (Group 4). With platelet labelling there were positive platelet accumulation index trends in the 30-min adenosine diphosphate group (Group 1). Adenosine diphosphate also produced platelet aggregation in platelet-rich porcine plasma. Only adenosine diphosphate (an intermediate platelet agonist) showed signs of platelet activation when considering all platelet parameters. The model should be further evaluated with different bolus doses of adenosine diphosphate, but may be used to evaluate if gas bubbles introduced into the circulation (as with decompression sickness), or possibly if clinical drugs, might produce platelet activation in vivo.

Introduction

In order to investigate if decompression sickness involves platelets, a platelet activation model was adapted and evaluated. The model had been used to study lung platelet trapping due to lipopolysaccharide (LPS), grafts, trauma and dialysis [1—4]. In investigating decompression sickness (DCS) our aim is to study possible platelet activation due to circulating gas bubbles. Gas bubbles activate porcine and human platelets in vitro [5,6]. Porcine platelets are more similar in biochemical properties to human platelets than platelets from several non-primate species [7—9]. Due also to general cardiovascular and pulmonary physiological similarities, such a pig model should then be of value in evaluating substances thought to activate human platelets [10,11]. Effects of adenosine diphosphate (ADP, an intermediate platelet agonist) and platelet activating factor (PAF, a weak platelet agonist) were studied in order to evaluate the model. Previous studies report significant early platelet aggregate formation and haemodynamic changes due to ADP [12,13]. This was confirmed in our pilot studies. We decided to study short- (5 min) and long-term (30 min) changes.

Materials and methods

The Norwegian Animal Research Authority had approved the study.

Animals

24 out-bred Norwegian Landrace pigs (and additional 7 pilot subjects) from Stend Agricultural School, Stend, Bergen, Norway, of both sexes and of conventional microbiological status, aged 3—4 months (17—42 kg), were housed at the school or at the Vivarium, Haukeland University Hospital, Bergen, Norway together with their siblings. During an acclimatization period of 1—2 days before the experiments each pig was kept in a separate pen (2×2 m, 2—4 pigs per room) at 20—23 °C on pine chip bedding and fed Svinefor 3 FK or Format Kombinorm sur (FK) with tap water ad libitum.

Pilot studies, animal grouping

In pilot studies four pigs received different doses of ADP (21.7—25—25—50 mg/kg) and three pigs different doses of PAF (0.04—0.4—4 μg/kg). 24 pigs were included in the actual study: 12 pigs each received a 20-ml saline suspension of ADP (Sigma® Prod.Cat. A-6521, 25 mg/kg) as a 5-min intravenous infusion provided by an iv. pump (IVAC®). Group 1 ($n = 6$) was studied for 30 min before sacrifice. Group 3 ($n = 6$) was sacrificed immediately after the infusion was completed. 12 pigs each received a similar saline suspension of PAF (Sigma® Prod.Cat. P-9525 0.4 μg/kg). Group 2 ($n = 6$) and Group 4 ($n = 6$) were studied for 30 and 5 min, respectively.

Anaesthesia levels, body temperature, heat loss prevention

Responses to painful stimuli were evaluated every 5 min and graded as none = 0, slight = 1 or forceful = 2. Any response graded 2 was deemed as insufficient anaesthesia implying need for additional anaesthetics [14]. Rectal temperature was measured continuously, with each animal covered up with a woolen blanket and with an isolating mattress (H.Hansen®) underneath to minimize heat loss.

Catheterization Day 1

An indwelling catheter (Charrière, size 8) supplied with a 3-way stopcock (Viggo®) was placed into the left external jugular vein and tunnelled subcutaneously to the animal's back on Day 1. A bolus of 20 ml of sterile saline (0.15 M NaCl) was infused after completion to avoid occlusion. General anaesthesia