Vet Res (1997) 28, 489-502

Original article

Morphological alterations of blood platelets induced by platelet activating factor (PAF) and partial inhibition by ketoprofen in calves

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(Received 17 March 1997; accepted 26 June 1997)

Summary — The influence of platelet activating factor (PAF) was investigated in vivo on the ultrastructure of bovine platelets, and on the platelet count. The effect of an intravenous administration of ketoprofen (a non-steroidal anti-inflammatory drug) pretreatment followed by PAF infusion was also observed in a group of six healthy male Friesian calves. PAF infusion alone caused a moderate thrombocytopenia, which peaked one minute post challenge and returned to levels not significantly different from control after 30 min. Electron microscopy revealed that after PAF infusion, platelets lost their lentiform shape and became irregular, with many pseudopods. Their microtubules became impossible to distinguish. The numbers of alpha granules and dense bodies were significantly decreased. Glycogen particles became rare or even disappeared. Giant platelets occasionally appeared. The Golgi apparatus was more often visible and the number of mitochondria was significantly increased. Ketoprofen pretreatment lowered PAF-induced thrombocytopenia and decrease in the number of dense bodies. Under these conditions, the Golgi apparatus was rarely visible and giant platelets were not observed. These results showed that the morphological ultrastructure of blood platelets in bovines were modified following PAF infusion and that ketoprofen pretreatment before PAF infusion provided partial protection, limiting the extent of the morphological alterations and maintaining a normal platelet count.

platelet / bovine / ketoprofen / blood / platelet-activating factor

Résumé — Altérations morphologiques des plaquettes sanguines induites par le facteur d'activation plaquettaire (PAF) et inhibition partielle par le kétoprofène chez le veau. L'influence in vivo du facteur d'activation des plaquettes (PAF) sur l'ultrastructure des plaquettes bovines ainsi que sur le comptage de ces plaquettes a été recherchée. Les effets de l'administration intraveineuse

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du kétoprofène (drogue anti-inflammatoire non stéroïdiene) suivie d'infusion de PAF ont également été observés dans un groupe de six veaux sains mâles de race Frisonne. L'infusion de PAF a causé une thrombocytopénie modérée, qui a atteint son sommet 1 minute après l'infusion et qui est revenue à une valeur non significativement différente du contrôle après 30 minutes. L'observation au microscope électronique a révélé qu'après l'infusion de PAF, les plaquettes ont perdu leur forme lenticulaire et sont devenues irrégulières, avec plusieurs pseudopodes ; les microtubules étaient impossibles à distinguer ; le nombre de granules alpha et de corps denses a significativement diminué ; les particules de glycogène étaient rares ou ont même disparu ; des plaquettes géantes sont occasionnellement apparues ; l'appareil de Golgi était rarement visible et le nombre des mitochondries a significativement augmenté. Le prétraitement avec le kétoprofène a limité la thrombocytopénie induite par le PAF et a empêché la diminution du nombre de corps denses ; l'appareil de Golgi était rarement visible et les plaquettes géantes n'étaient pas visibles. Ces résultats montrent que l'ultrastructure morphologique des plaquettes sanguines chez le veau est modifiée après l'infusion de PAF. Le prétraitement avec le kétoprofène avant l'infusion de PAF produit une protection partielle contre les altérations morphologiques et la thrombocytopénie induite par le PAF.

plaquette / bovin / kétoprofène / sang / facteur d'activation des plaquettes

INTRODUCTION

Ketoprofen or [(±) 2-(3-benzoylphenyl)] propanoic acid is a non-steroidal anti-inflammatory drug (NSAID) that has been widely used in human medicine for more than 20 years, as an antiinflammatory and analgesic agent (Veys, 1991). Ketoprofen is also used for the treatment of musculoskeletal conditions in horses, dogs and calves (Jaussaud et al, 1993; Landoni et al, 1995). Recently, a report suggested that ketoprofen significantly improved recovery from clinical mastitis in dairy cows (Shpigel, 1994). It contains a single chiral centre and two isomeric forms, the S (+) and R (-) enantiomers. The product available for use in veterinary medicine is the racemic mixture (Landoni et al, 1995). The principal mechanism of NSAID action is considered to be the inhibition of prostanoid biosynthesis through a cyclo-oxygenase blockade (Vane, 1971).

In calves, ketoprofen inhibits ex vivo platelet activating factor (PAF)-induced irreversible aggregation of platelets (Bastos da Silva et al, 1997). However, the acetylsalicyclic acid (aspirin) does not appear to be a universal inhibitor of platelet function. Bovine platelets are insensitive to the inhibitory effects of aspirin, regardless of the aspirin concentration used (Gentry et al, 1989). In humans, inhibition of the granule release reaction by platelets is significantly stronger in the presence of ketoprofen than indomethacin (Migne et al, 1983). In addition, ketoprofen affects platelets by causing a readily reversible inhibition of aggregation induced by ADP, epinephrine and collagen in vitro and by slightly increasing the bleeding time (Gandini et al, 1983).

Platelet activating factor (PAF), the common name for 1-*O*-alkyl-2-*O*-acetyl-sn-glyceryl-3-phosphorylcholine, is a phospholipid produced by the endothelium, myeloid lineage cells, and other cells that interact with platelets and induce aggregation (Prescott et al, 1990). The effects of PAF on platelets have been studied mainly under in vitro conditions. Some investigators have demonstrated that bovine platelets are very sensitive to PAF (Liggitt et al, 1984; Bondy and Gentry, 1989; Bastos da Silva et al, 1996). In platelet-rich plasma, the addition of low concentrations of PAF produce a reversible aggregation and higher concentrations of PAF induce an irreversible aggregation (Bastos da Silva et al, 1996). In addition, PAF causes increases in the cytosolic calcium concentration, phosphatidylinositide hydrolysis, protein phosphorylation, shape change, and granule release in human platelets (Demopoulos et al, 1979; McIn-

tyre and Pollock, 1983). Moreover, the release of dense granule contents following PAF stimulation appears to require an intact cyclo-oxygenase pathway (Chignard, 1987). In most cell types, the concentration of PAF existing in the resting cell is low, and PAF formation is dependent upon the presence of a membrane stimulus (Meade et al, 1991). The extent to which bovine platelets themselves can produce PAF is unclear. It is now recognized that some of the biological activities of PAF are secondary to platelet activation (Lellouch-Tubiana et al, 1988; Coyle et al, 1990) and that the platelet may act as an inflammatory cell in its own right (Page, 1989).

When administered in vivo, PAF is the single most potent endogenous agent known to produce the shocked state, its effects depend on the species studied (Feuerstein and Hallenbeck, 1987). PAF also produces broncho-constriction, profoundly increases pulmonary vascular resistance, decreases cardiac output and peripheral vaso-constriction and produces an increase in vascular permeability. The increase in pulmonary vascular resistance, elevation of pulmonary arterial pressure, and bronchoconstriction leads to progressive hypoxia and right ventricular failure (William and Henderson, 1991; Mathias and Behrens, 1992; Maxwell, 1993; Félez et al, 1994).

The structure of platelets has been studied in many species. No study of the ultrastructure of bovine platelets stimulated by intravenous administration of PAF has yet been reported. To date no study has been published to show the type of protection that a non-steroidal anti-inflammatory drug (ketoprofen) could provide to bovine blood platelets before a PAF challenge. A definite need for such a study is obvious, when one considers the range of possible PAF effects including involvement in various inflammatory, respiratory and cardiovascular disorders, and which are due to its high potency to activate bovine platelets.

The aim of this study was (1) to determine in vivo the influence of PAF on the ultrastructure of bovine platelets and the platelet count; and (2) to check if this influence could be modified by ketoprofen, a non-steroidal anti-inflammatory drug.

MATERIALS AND METHODS

Materials

The hexadecyl species of PAF was obtained from Bachem Feinchemikalien (Bubendorf, Switzerland) and stored as stock solution (1 mg/mL) in ethanol at – 20 °C until required. For in vivo studies PAF solution was dissolved on the day of the experiment in saline (NaCl 0.9% solution). The following pieces of equipment and chemicals were used for the electron microscope analysis: Eppendorf microfuge (model 320, Brinkman Instruments, Inc Westbury, New York, USA); EM bed 812 (20 g) nadic methyl anhydride (NMA) 10 g; specially distilled (DDSA) 10 g, 2, 4, 6-tri (dimethylaminomethyl)phenol (DMP) 0.4 g, Electron Microscopy Science (Fort Washington, Pennsylvania, USA). Glutaraldehyde 25% EM grade, Agar Scientific Ltd (Stansted, Essex CM 248DA, UK), Osmium tetroxide, E Merck PA (Darmstadt, Germany), 1.2-epoxypropane, Agar Scientific Ltd (Stansted, UK), Uranyl acetate (Analar, BDH chemicals Ltd, Poole, UK), Lead citrate (Ultostain 2, Leica, Austria), Zeiss EM 910 transmission electron microscope, 80 kV, Carl Zeiss nv-sa (Oberkochen, Germany). Ketofen® 10% (form: ketoprofen 10 g, benzyl alcohol 1 g, L-Arginin–citric acid monohydrate, Aqua ad inject qsp 100 mL) was supplied by Rhône Mérieux SA, Brussels, Belgium.

Animals

Six healthy Friesian male calves, weighing 314 ± 11 kg (mean ± SD), aged 10–11 months were used for this study.

Experimental design

A catheter was introduced into the jugular vein before starting the experiment. PAF solution (1 mg/mL) was added to a solution of NaCl (0.9 %) 5 min before the start of the PAF administration.

In the first study, PAF infusion was performed intravenously at a dose of 50 ng/kg/min for 5 min in the six calves. In the second study, these animals received the second challenge of PAF between 7 and 21 days later.

For the second study racemic ketoprofen (Ketofen® 10%; Rhône Mérieux, Belgium) pretreatment was infused at a dose rate of 3 mg/kg into the jugular vein as a bolus. Ten minutes after the ketoprofen pretreatment, the solution of PAF was administered intravenously at a dose of 50 ng/kg/min for 5 min to each calf.

For each study blood samples were collected for analysis under the electron microscope and for counting the total number of platelets.

Platelet count

Blood samples for the total platelet count were collected after the end of the PAF infusion in 'vacutainer' tubes containing 7.5% EDTA and were obtained before PAF infusion and 1, 3, 5, 15, 30 min, and 1, 2, 3 h after the end of PAF infusion. After collection, these samples were heated at room temperature (18–20 °C) for a maximum of 3 h. The total platelet count was determined by the haematology analyser Technicon H_2 (Bayer PA, Diagnostic Division, Wemmel, Belgium).

Preparation of platelets for study in the electron microscope

Blood samples were collected in 5 mL 'vacutainer' tubes containing 0.5 mL of 3.8% trisodium citrate before and 1, 3, 5 min after the end of PAF infusion for electron microscopic studies. Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 270 *g* for 20 min at 10 °C, and collecting the upper two thirds of the PRP. The platelet counts in the PRP were between 300 and 766 × 10^3 per µL for all the platelet preparations. After determining the platelet count, aliquots of PRP (1 000 µL) were placed in an Eppendorff microfuge and fixed with 0.1% glutaraldehyde in phosphate buffered saline (V/V) for 15 min. After 15 min the samples of PRP were centrifuged at 270 *g* for 5 min at 18 °C. The supernatant fixative was removed, replaced with 2.5% glutaraldehyde and incubated for 1 h. The pellets were washed three times with phosphate buffered saline (PBS, PO4 buffer 0.05 mol/L - pH 7.4 - NaCl 0.85%) for 10 min at 4 °C, and post fixed with 1% aqueous osmium tetroxide for 1 h at 4 °C. After post fixation the pellets were washed with distilled water at 4 °C. All samples were dehydrated with graded ethanols (70% for 15 min at 4 °C, 95% at room temperature, three times for 5 min, methanol at room temperature, three times for 20 min) and embedded in a grade series of EM bed 812 combined with 1.2-epoxypropane (1/2, 1/1, 2/1 at room temperature for 1 h). After 1 h the samples were embedded in pure EM bed 812 at room temperature overnight and heated for 48 h in pure EM bed 812. Thin sections were contrasted with uranyl acetate (0.5 g in 12.5 mL distilled water + methanol 1/1) for 10 min followed by lead citrate for 1 min. Sections were examined with a Zeiss 910 transmission electron microscope.

Ultrastructure

Platelets sectioned in the equatorial plane or perpendicular to the equatorial plane were analysed qualitatively and quantitatively. One hundred sectioned platelets were observed under the electron microscope, and were analysed for each animal ($n = 6$) and for each time ($n = 4$). The platelet profiles were observed with respect to shape (regular, irregular), number of alpha granules (0–12 or 13–24), dense bodies (with 0 or 1–3) and mitochondria (number visible). The microtubules were classified with respect to the possibility of being seen (visible microtubules) or not (invisible microtubules). The glycogen particles were analysed with respect to their distribution (normal or abnormal). The first group of platelet sections was considered normal when the particles of glycogen were aggregated or clumped together, and the second one abnormal where the glycogen particles were dispersed, rare or absent.

Statistical analysis

Results are expressed as the mean values ± standard error of the mean (SEM) and were examined for statistical significance using analysis of variance. Multiple comparisons of means were performed by the Tukey Test. P values under 0.05 were considered statistically significant (Vieira, 1985).

RESULTS

Platelet counts

The normal platelet count was in the range 355–540 ($\times 10^3/\mu L$) and the mean was 452 ± 84 ($\times 10^3/\mu L$) of blood. In the first study (PAF alone) the blood platelet count was significantly decreased beginning 1 min and lasting until 15 min after the end of PAF infusion. Thrombocytopenia was maximal 1 min after the PAF infusion. The number of platelets in circulation returned to levels not significantly different from control values 30 min after the end of the PAF infusion. For the second study, where ketoprofen was injected as a pretreatment, the number of platelets decreased significantly between 1 and 3 min only following the PAF infusion. These values returned to levels not significantly different from control values 5 min following the PAF infusion (table I).

Morphological studies

PAF infusion alone

Before PAF infusion (control)

We observed that bovine blood platelets had various shapes and sizes (fig 1A, B, C). As shown in table II, 81% of the control platelet profiles showed a regular shape. The alpha-granules were usually round and large. We found from 1 to 26 alpha-granules per platelet section. The dense granules were round and characterized by an extremely electron-dense core or body that was usually positioned near one side granule (fig 1B, C). Mitochondria were occasionally visible (fig 1A). Only 20% of the platelet sections showed from one to three mitochondrial profiles. All platelet sections showed

Table I. Blood platelet number ($\times 10^3/\mu L$) before and after PAF infusion (50 ng/kg/min for 5 min), preceded or not by an intravenous administration of ketoprofen (3 mg/kg) in calves (mean ± SEM; $n = 6$).

	Without ketoprofen	*With ketoprofen*
Control		
Before PAF	452 ± 76	
Before ketoprofen	ND	445 ± 60
After ketoprofen (10 min)	ND	402 ± 55
After PAF		
1 min	54 ± 28*	60 ± 14*
3 min	131 ± 71*	131 ± 30*
5 min	188 ± 80*	293 ± 108
15 min	239 ± 82*	354 ± 66
30 min	306 ± 56	390 ± 71
1 h	372 ± 97	ND
2 h	418 ± 79	ND
3 h	445 ± 78	ND

* Significantly different from control with $P < 0.05$. ND: not determined.

visible microtubules (fig 1A, B, C). The normal glycogen particles were visible in about 99% of the platelet profiles. The open canalicular system (OCS), dense tubular system (DTS) and lysosome were morphologically indistinguishable. A Golgi apparatus was occasionally seen in a platelet.

After PAF infusion

The ultrastructure in bovine platelets was significantly altered after the end of the PAF infusion (table II). Using electron microscopy, we observed that the platelets showed shape changes (figs 2, 3, 4). Pseudopod formation was obvious (figs 2A, B, C, 3C, 4C). The proportion of platelets in a given section with regular shape was significantly decreased by 63% while the proportion of irregularly formed platelets was significantly increased by 278%. Occasionally giant platelets were visible (figs 3B, C, 4C). The microtubules became impossible to distinguish at the platelet periphery (figs 2B, 3C, D, 4A). The alpha-granules became larger, more elliptical, sometimes rare (figs 2A, B, C, 3A, B, D, 4A). Degranulation of platelets was observed as shown in fig 4A. Occasionally endocytosis or exocytosis was observed in the external coat membrane (figs 2A, 3C, D). The proportion of platelets having 13 to 24 alpha-granules per section was significantly decreased, and had not returned to control values 5 min after PAF infusion. The proportion having 0 to 12 alpha-granules per platelet section was significantly increased by 11%. The number of dense bodies for the proportion of platelets with 0 dense bodies increased by about 140% at 3 min following the PAF infusion, but the group with 1 to 3 dense bodies per platelet section decreased by about 49%. The glycogen particles were dispersed, rare or even disappeared as shown in figures 2, 3 and 4. This alteration was significantly increased by 3 085% for the abnormal group 5 min after the PAF infusion. Mitochondria became more visible with more than four mitochondria being observed in each platelet section (figs 4B, C). The Golgi apparatus was also more often visible (fig 3A).

Table II. Effect of intravenous administration of ketoprofen (3 mg/kg) and PAF infusion (50 ng/kg/min for 5 min) on morphological ultrastructure of blood platelets in six calves (mean ± SEM, 100 platelets sections/animal/time).

	Control (0 min)	*1 min*	*3 min*	*5 min*
PAF only				
Shape (% regular)	81 ± 5	30 ± 7*	40 ± 3*	45 ± 4*
Alpha-granules (% 0–12)	89 ± 2	97 ± 1*	98 ± 1*	97 ± 1*
Dense bodies (% with 0)	26 ± 4	59 ± 5*	63 ± 5*	57 ± 9*
Microtubules (% visible)	100 ± 0	13 ± 3*	15 ± 6*	6 ± 3*
Glycogen particles (% normal)	99 ± 1	31 ± 19*	36 ± 6*	28 ± 15*
Mitochondria (%)	20 ± 3	37 ± 5*	50 ± 9*	56 ± 6*
Ketoprofen + PAF				
Shape (% regular)	80 ± 2	45 ± 5*	71 ± 3*	73 ± 1*
Alpha-granules (% 0–12)	86 ± 1	100 ± 0*	96 ± 2*	94 ± 2*
Dense bodies (% with 0)	59 ± 6	60 ± 5	44 ± 6*	41 ± 4*
Microtubules (% visible)	34 ± 4	58 ± 2*	14 ± 1*	21 ± 3*
Glycogen particles (% normal)	81 ± 2	59 ± 4*	87 ± 2*	95 ± 1*
Mitochondria (%)	19 ± 1	34 ± 3*	36 ± 2*	31 ± 3*

* Significantly different from control with $P < 0.05$.

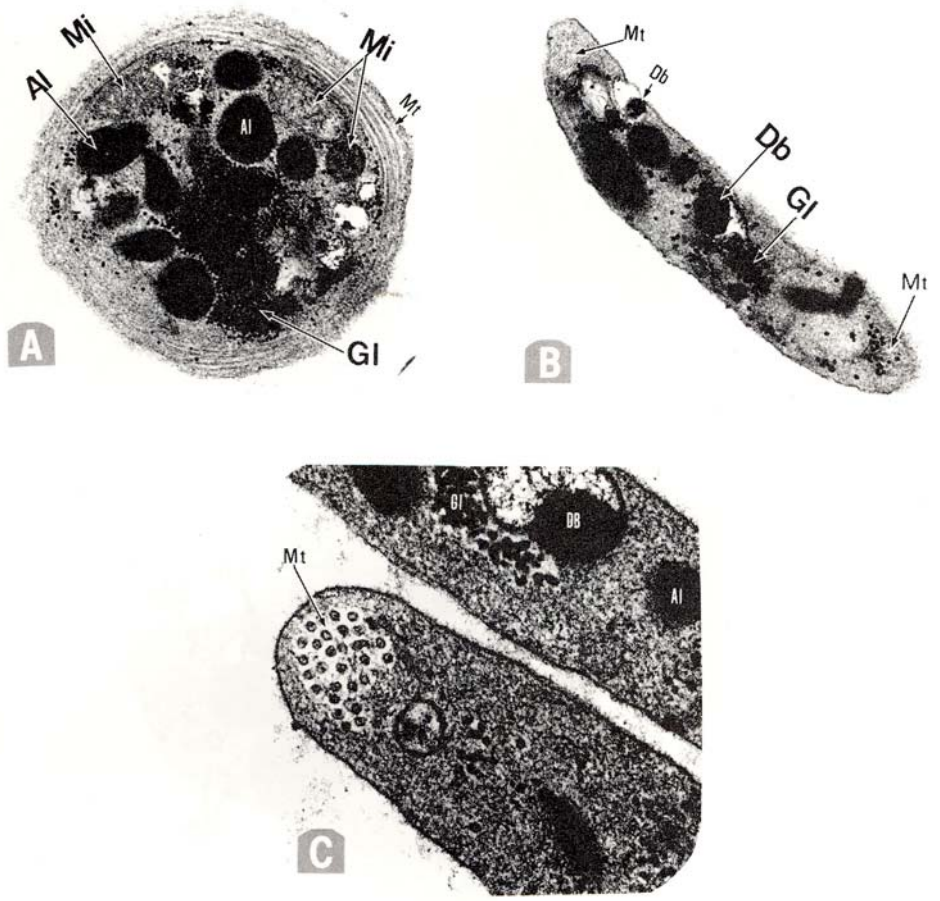

Fig 1. Electron micrograph of bovine platelets before PAF infusion showing: A - a round platelet, circumferential microtubules (Mt) coiled just under the surface membrane, which support the lentiform appearance of the unstimulated cell, alpha-granules (Al), mitochondria (Mi); A, B - glycogen particles (Gl) are clumped together; B - an ellipitical platelet with two dense bodies (Db); C - a marginal bundle of microtubules, the core of the dense body nearly fills the membrane-bound vesicle (A: × 12 500; B: × 25 000; C: × 20 000).

Ketoprofen treatment followed by PAF infusion

Before ketoprofen infusion (control)

We observed by means of electron microscopy that the profiles of platelets in controls for the second study had regular shapes (80%), and normal numbers of alpha-granules (86%) and mitochondria (19%). However, the number of dense bodies, visible microtubules and glycogen particles was different from the first study, because

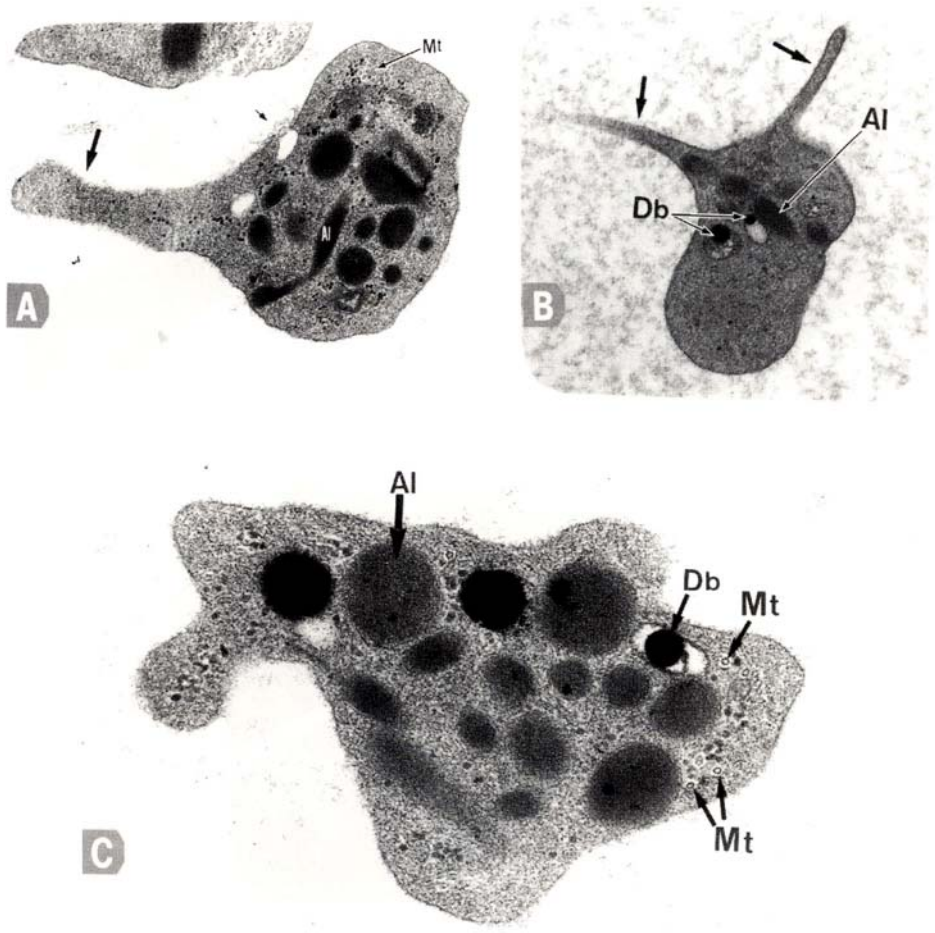

Fig 2. One minute after PAF infusion, electron micrograph of bovine platelets showing PAF-activated platelet: shape change (A - C); alpha-granules (Al) are ellipitical (A), sometimes small or rare (B and C); glycogen particles are more rare (A and C) or absent (B and D); A - exocytosis and endocytosis in the cellular membrane (arrow); B - the microtubules (Mt) are still evident at the platelet periphery (A), but not in the pseudopod (A and B) large arrows; C - three dense bodies in the platelet periphery (A: × 20 000; B: × 8 000; C: × 25 000).

the animals utilised in the second study had previously received one dose of PAF and these alterations persisted when experiment two was started (table II).

After ketoprofen followed by PAF infusion

As shown in table II, after 3 min of PAF infusion, ketoprofen pretreatment followed

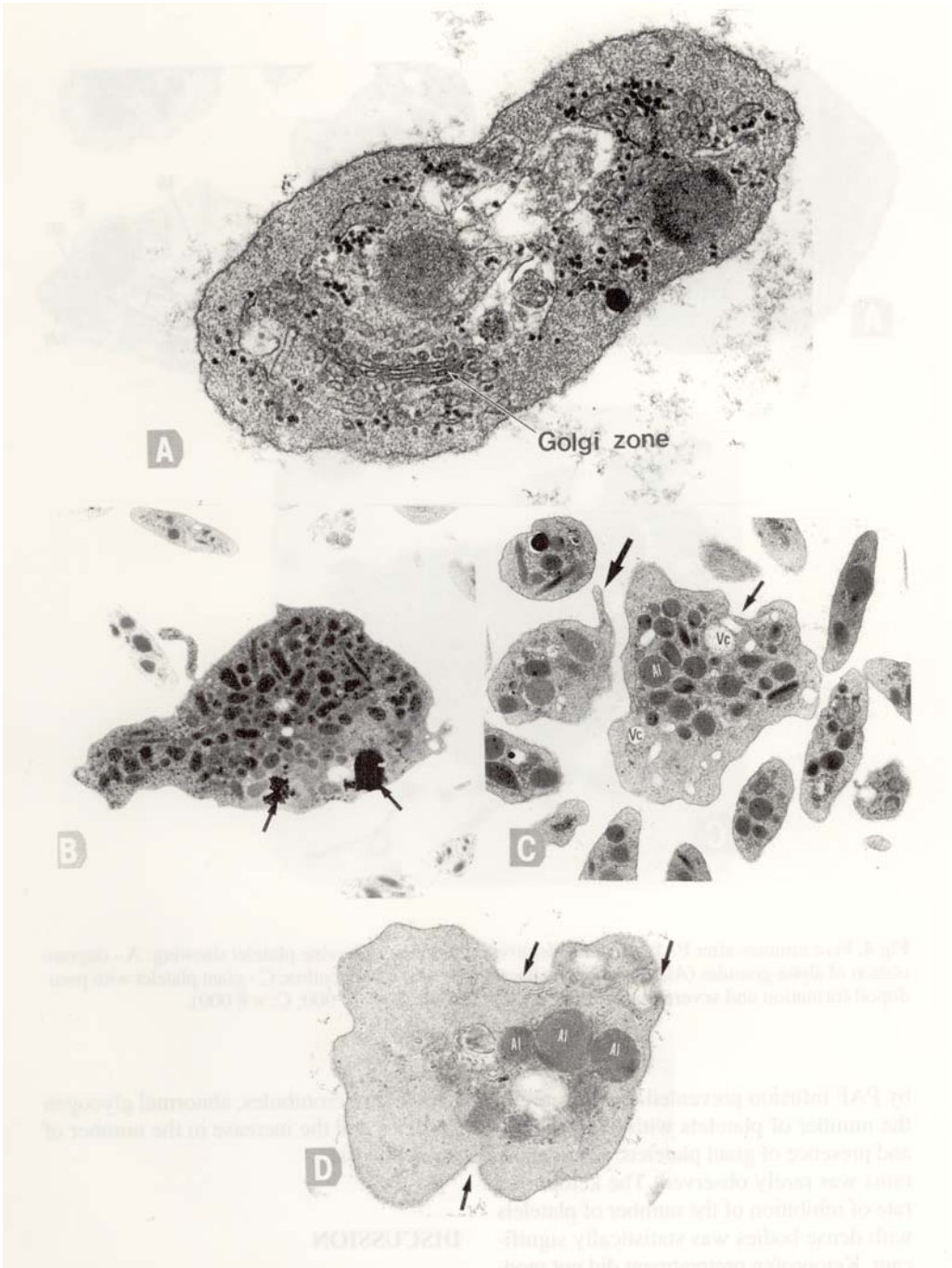

Fig 3. Three minutes after PAF infusion, electron micrograph of bovine platelets showing: A - Golgi apparatus appears; the alpha-granules are now rarely found and the glycogen particles are dispersed, × 20 000; B - giant platelet shows 156 alpha-granules, including two granules that present lead citrate-stained material, × 8 000 (arrows); C - vacuoles are more evident (Vc), occasionally giant platelets are located near to small platelets, pseudopods are free of microtubules (large arrow); C and D - the cellular membrane shows endocytosis (arrows) (C: × 12 500; D: × 25 000).

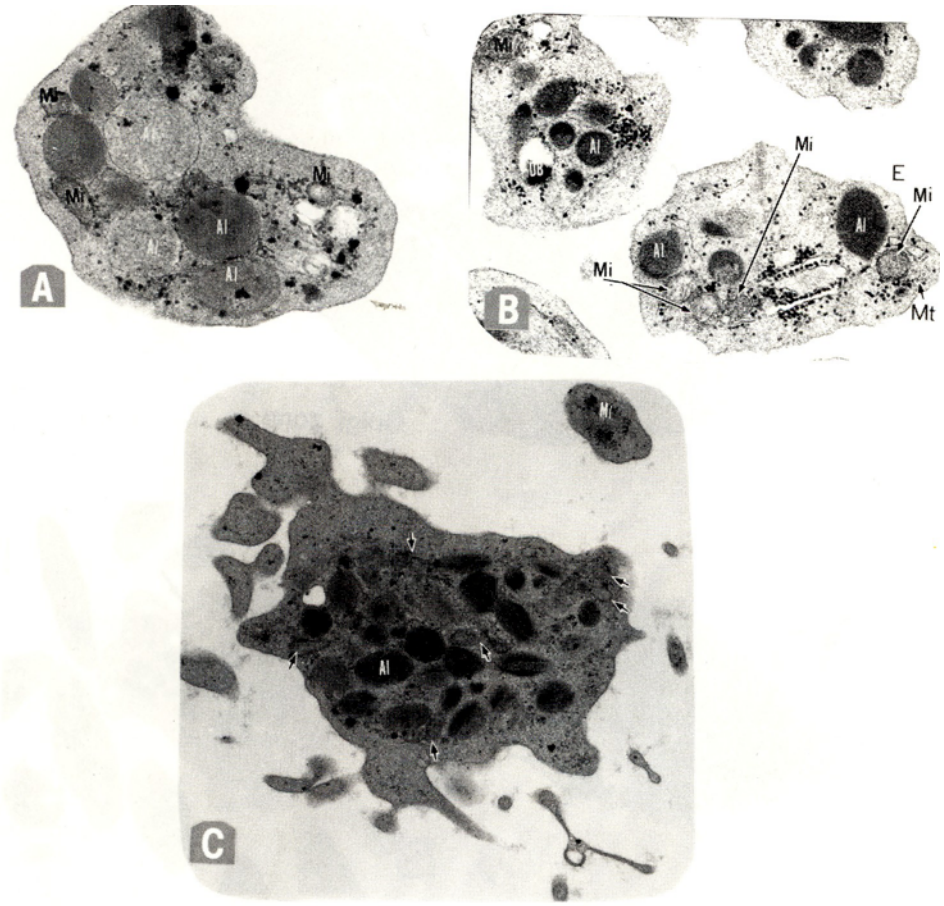

Fig 4. Five minutes after PAF infusion, electron micrograph of bovine platelet showing: A - degranulation of alpha-granules (Al); B - this cell appears with four mitochondria; C - giant platelet with pseudopod formation and several mitochondria (A: × 25 000; B: × 20 000; C: × 8 000).

by PAF infusion prevented the decrease in the number of platelets with dense bodies and presence of giant platelets. Golgi apparatus was rarely observed. The ketoprofen rate of inhibition of the number of platelets with dense bodies was statistically significant. Ketoprofen pretreatment did not modify some of the alterations induced after PAF infusion including the irregular shape, decrease in the number of alpha-granules, invisible microtubules, abnormal glycogen particles and the increase in the number of mitochondria.

DISCUSSION

PAF infusion reduced the number of circulating platelets. This meant that the population of platelets in controls was different

from the population following infusion with PAF. One may consider that the only platelets that remain in the circulation were those that were not able to aggregate. This rebound phenomenon (platelets returning to their initial conditions) could have resulted from a self-regulatory mechanism for restoring the platelet levels.

In this study, electron microscopy revealed that after PAF infusion, platelets lost their lentiform shape and became irregular, with many pseudopods. This response of the bovine platelets to PAF was similar to that observed in humans (White and Rao, 1983; White, 1987; Grouse et al, 1990; Gentry, 1992) during platelet activation, particularly in response to a strong agonist such as collagen or thrombin (White, 1987). The changes in surface contours are associated with a process of internal transformation (White and Rao, 1983). Although the reason why platelets undergo shape change is unclear, one possibility is that their electrostatic repulsion may be reduced without reducing the surface charge density. Thus, the tip of a deformed platelet can approach and make contact with a surface or a cell, more easily as most of the repulsive surface charge remains relatively distant (Coller, 1983). Another possibility is that morphological alterations, such as becoming spherical, budding, and pseudopod formation can be attributed to the disintegration of the microtubule structure (Jain, 1993).

We also observed that the microtubules were less visible or impossible to distinguish after the PAF infusion. This response suggested that the platelet microtubular system became less resistant to deformation after a PAF infusion. It is possible that the microtubules depolymerize in platelets following activation (White, 1994; Ware and Coller, 1995).

This work demonstrated that PAF induced depletion in alpha-granules and dense bodies in bovine platelets. These results were similar to those demonstrated in vitro when PAF induced reversible platelet aggregation in cattle (Bastos da Silva et al, 1996). The Golgi apparatus was more often visible after the PAF infusion. This suggested that immature platelets were released into the blood stream.

Bovine platelets have sizeable stores of glycogen that can often be seen by means of electron microscopy (see fig 1A). The present investigation showed that the intravenous administration of PAF induced glycogen particles to be dispersed, rare or absent. The glycogen particles have an important function in platelet energy metabolism. According to Akkerman and Holmsen (1981), glycogen can be broken down into glucose 1-phosphate. Platelets can also take up glucose from their surrounding medium. Both sources of glucose can be converted into glucose 6-phosphate, which can then be used for glycolysis or the hexose monophosphate shunt. In this work, we demonstrated that the number of mitochondria in the platelet profiles increased after 5 min of PAF infusion. This means that the increase in the number of platelet mitochondria is the consequence of megakaryocyte activation as mitochondrial duplication needs nuclear transcription. Thus, platelet mitochondria are involved in oxidative energy metabolism, and they can replicate themselves, each time a cell needs increased quantities of ATP (Guyton, 1980). Platelet stimulation is accompanied by a marked increase in both glycolytic activity and oxidative ATP production, perhaps owing to the abrupt decrease in ATP that occurs with platelet activation or the increase in cytoplasmic pH (Akkerman and Verhgoeven, 1987).

Occasionally, we observed giant platelets after PAF infusion. It has been suggested that large platelets are hyperfunctional (Martin et al, 1983). Larger platelets are often found in situations involving platelet destruction or consumption, compared with platelet sequestration or production failure,

in which small platelets are a more common finding. In fact, an increase in the mean platelet volume (MPV) has been attributed to megakaryocyte stimulation (Corash et al, 1987; Thompson and Jakubowski, 1988).

We observed that the platelet sections examined before ketoprofen pretreatment (control), for those animals that had received the two doses of PAF with a 7- to 21-day interval between doses, kept the alterations induced by PAF infusion such as dispersal or absence of glycogen particles, decrease in the numbers of dense bodies and the values for invisible microtubules. We believe that the observed changes were caused by PAF infusion as this agrees with an earlier study in calves, which showed that the whole platelet ultrastructure had normal control values before PAF infusion (Bastos da Silva et al, 1997, unpublished data). Thus, the involvement of PAF in this mechanism is still unclear. However, the different percentages for the (second) study with ketoprofen pretreatment after PAF alone on these above mentioned ultrastructures were smaller than for the (first) study with PAF alone. Since no similar studies have been published, it is not possible to compare the effect of ketoprofen on the ultrastructure of platelets in bovine with other NSAIDs.

We have demonstrated in this study that ketoprofen pretreatment prevents the moderate thrombocytopenia 5 min after PAF infusion, probably resulting from the inhibition of a reversible process of intravascular platelet aggregation. In those animals that received PAF without pretreatment, the number of platelets significantly decreased for 15 min after the PAF infusion.

In this work, electron microscopy revealed that ketoprofen pretreatment before a PAF infusion also provided partial protection of the platelet ultrastructure, because the Golgi apparatus was rarely visible and we did not observe giant platelets. However, in the first study without ketoprofen pretreatment, these structures were more visible. In addition, the number of dense bodies returned to control values with ketoprofen pretreatment in spite of the fact that the experimental animals had previously received one dose of PAF. The inhibition of PAF-induced effects by ketoprofen pretreatment was statistically significant for these values. This may have been due to the prostanoid biosynthesis inhibition of platelets caused by the drug rac-ketoprofen. The (S)-enantiomer of ketoprofen is responsible for the cyclo-oxygenase inhibitory activity in human and rabbit platelets (Hayball et al, 1992; Suesa et al, 1993). According to Suesa et al (1993), the inhibition of ketoprofen is exclusively due to its dextrorotatory enantiomer. Moreover, only very recently Landoni et al (1995) have reported in calves that ketoprofen inhibited serum thromboxane B_2, exudate prostaglandin (PGE_2) synthesis and exudate β-glucuronidase release as well bradykinin-induced oedematous swelling. However, there was no effect on exudate leucotriene (LTB_4) concentrations.

It has become evident in this work that electron microscopy clearly reveals blood platelet alterations in calves after PAF infusion. These alterations last longer than the physiological recovery period of the experimental animals (Bastos da Silva et al, unpublished data). PAF induces moderate thrombocytopenia, probably resulting from a reversible process of intravascular platelet aggregation. Ketoprofen pretreatment provides a partial protection for both morphological alterations and platelet count.

ACKNOWLEDGMENTS

The authors thank F Herion, Y Cooghe, C Uystepruyst, L Fery and M Leblond for their technical assistance, P De Moor for the statistical analysis and JF Bradfer for the excellent technical assistance with electron microscopy. Financial support from the Rhône Mérieux (Lyon, France) and provision of Ketofen® 10% (Brussel, Belgium) is gratefully acknowledged.

M Bastos da Silva is a recipient of grant from CNPq and the Faculty of Veterinary Medicine (FCAP), Belém, Pará of Brazil.

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