

Effect of BM-573, a dual thromboxane synthase inhibitor and TP receptor antagonist, in a porcine model of acute pulmonary embolism.

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Abbreviations:

BM-573, *N*-terbutyl-*N*'-[2-(4'-methylphenylamino)-5-nitro-benzenesulfonyl]urea;

TP receptor, thromboxane receptor; **TXA₂**, thromboxane A₂; **TXB₂**, thromboxane B₂;

PAP, pulmonary artery pressure; **RV**, right ventricular; **E_{es}**, end-systolic elastance;

E_a, arterial elastance; **R₁**, pulmonary characteristic resistance; **R₂**, distal pulmonary vascular resistance; **C**, total pulmonary compliance; **L**, inductance; **PaO₂/F_iO₂**,

arterial oxygen tension to inspired oxygen fraction ratio; **PaCO₂**, arterial carbon dioxide tension.

Section: cardiovascular

Abstract

The aim of this study was to evaluate the effect of BM-573, a dual thromboxane A₂ synthase inhibitor and receptor antagonist, on the hemodynamic response to acute pulmonary embolism. Six anaesthetised pigs were infused with placebo (Placebo group) and compared with 6 other pigs receiving a continuous infusion of BM-573 (BM group). Pulmonary embolization with 0,3 g/kg autologous blood clots was carried out 30 minutes after the start of the infusion. Right ventricular pressure-volume loops were recorded using a conductance catheter and end-systolic ventricular elastance was periodically assessed by varying right ventricular preload. Pulmonary vascular properties were studied by use of a four-element windkessel model. Hemodynamic data including assessment of right ventricular-arterial coupling were collected at baseline, and every 30 minutes for 4 hours. Blood samples were collected to assess gas exchange, thromboxane A₂ and prostacyclin plasma levels, and to evaluate platelet aggregation. Mean pulmonary arterial pressure in the Placebo group increased significantly more than in the BM group, mainly because of an additional increase in pulmonary vascular resistance. Arterial and end-systolic ventricular elastances increased also more in the Placebo group, while right ventricular efficiency decreased. BM-573 prevented both platelet aggregation induced by U-46619 or by arachidonic acid, and thromboxane A₂ overproduction, while prostacyclin liberation was preserved. Oxygenation was however not significantly improved.

We conclude that in this animal model of acute pulmonary embolism, infusion of BM-573 reduced pulmonary vasoconstriction. As a result, right ventricular-vascular coupling values were maintained at a maximal efficiency level.

Acute pulmonary embolism with hemodynamic instability still carries a high mortality rate and is associated with sudden death since up to 90% of the non-survivors succumb within two hours after the onset of symptoms (Stein and Henry, 1995). Abrupt increase in pulmonary vascular resistance involving mechanical obstruction is worsened by acute liberation of vasoconstricting mediators, leading to acute right heart failure and ventricular-vascular uncoupling (Smulders, 2000).

There is growing evidence that thromboxane A₂ (TXA₂), one of the end products of arachidonic acid metabolism and a potent proaggregating and vasoconstrictor agent, may be involved in the early pathogenesis of pulmonary embolism (Klotz et al., 1984; Oates et al., 1988; Smulders, 2000). TXA₂ liberation occurs in the very first minutes following embolism and its degree of production correlates with the risk of mortality in experimentally induced pulmonary embolism (Reeves et al., 1983; Utsonomiya et al., 1982). TXA₂ dominates early vasomotor response, accounting for the early hemodynamic impairment, while prostacyclin is more active in later course (Reeves et al., 1983; Smulders, 2000). Attempts made in the past to modulate TXA₂ production using cyclooxygenase inhibitors showed an attenuation of hemodynamic response to pulmonary vascular embolization when the drug was used as pre-treatment (Johnson and Malik, 1985; Perlman et al., 1987; Utsonomiya et al., 1982). Other results were rather disappointing because prostacyclin production was also impaired, worsening hypoxemia or increasing pulmonary vascular resistances (Delcroix et al., 1992; Hofman and Ehrhart, 1987; Johnson and Malik, 1985). Furthermore, pure TXA₂ synthase inhibitors efficacy was limited by an incomplete blockade at the dosage used and because of the accumulation of endoperoxide H₂ (PGH₂), a TXA₂ precursor, which exerts similar biological effects by occupying

common receptors (FitzGerald, 1991; Fukushima and Kobayashi, 1986; Ishihara et al., 1986; Lelcuk et al., 1987; Rolin et al., 2001). Therefore, dual compounds acting as both thromboxane synthase inhibitors and receptor antagonists such as ridogrel were tested and results suggested a potential interest in many pathophysiological states (Dogne et al., 2000a). Nevertheless, the effects of such a drug have never been experimented in an animal model of acute pulmonary embolism.

Torasemide, a loop diuretic, is able to slightly relax the dog coronary artery precontracted by TXA₂ (Uchida et al., 1992). Replacement of its pyridine ring with a nitrobenzene improves TXA₂ antagonism and reveals TXA₂ synthase inhibitory potency (Dogne et al., 2001), while the presence of a *tert*-butyl chain on the distal nitrogen of the sulfonylurea function is propitious for a high TXA₂ antagonism activity (Dogne et al., 2000b). These pharmacomodulations led to the development of BM-573 (*N*-*tert*-butyl-*N'*-[2-(4'-methylphenylamino)-5-nitro-benzenesulfonyl]urea), a novel dual TXA₂ synthase inhibitor and receptor antagonist. This product has been shown to prevent human platelet aggregation and thromboxane synthesis induced by arachidonic acid and to relax rat aorta artery precontracted with U-46619, a stable TXA₂ agonist (Rolin et al., 2001). In pigs, BM-573 completely antagonised pulmonary hypertensive effects of U-46619 (Lambermont et al., 2003) and reduced the early phase of pulmonary hypertension in a model of endotoxic shock (Lambermont et al., 2004). Finally, BM-573 was able to protect pig from myocardial infarction induced by coronary thrombosis (Rolin et al., 2003).

Given the role of TXA₂ in the pathogenesis of pulmonary vascular abnormalities in the acute phase of pulmonary embolism, the present study was undertaken to investigate the effects of this new dual inhibitor in an animal model of acute pulmonary embolism. Specifically, we estimated the effect of a pretreatment with BM-

573 on pulmonary hemodynamics, on TXA₂ and prostacyclin production, on platelet aggregation and, finally, on gas exchange evolution.

Material and methods

All experimental procedures and protocols used in this investigation were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Liege. They were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

Surgical preparation

Experiments were performed on 22 healthy pure pietran pigs of either sex weighing 20 to 30 kg. The animals were premedicated with intramuscular administration of ketamine (20 mg/kg) and diazepam (1 mg/kg). Anesthesia was then induced and maintained by a continuous infusion of sufentanil (0.5 µg/kg/h) and pentobarbital (5 mg/kg/h). Spontaneous movements were prevented by pancuronium bromide (0.2 mg/kg/h). After endotracheal intubation through a cervical tracheostomy, the pigs were connected to a volume cycled ventilator (Evita 2, Dräger, Lübeck, Germany) set to deliver a tidal volume of 10 mL/kg at a respiratory rate of 20 breaths/min. End-tidal CO₂ measurements (Capnomac, Datex, Helsinki, Finland) were used to monitor the adequacy of ventilation. Respiratory settings were adjusted to maintain end-tidal CO₂ between 30 and 35 mmHg while the inspired fraction of oxygen was 40%. The pulmonary trunk was exposed by means of medial sternotomy. A micromanometer-tipped catheter (Sentron pressure-measuring catheter, Cordis, Miami, FL) was inserted into the main pulmonary artery through a stab wound in the right ventricular outflow tract. A 14 mm diameter perivascular flow probe (Transonic Systems, Ithaca, NY) was closely adjusted around the main pulmonary artery 2 cm downstream of the pulmonary valve. The micromanometer-tipped catheter was manipulated so that the

pressure sensor was finally positioned very close to the flow probe. Left atrial pressure was measured with a micromanometer-tipped catheter inserted into the cavity through the left atrial appendage. Systemic blood pressure was monitored with a micromanometer-tipped catheter inserted into the descending thoracic aorta through the left femoral artery. A 7F, 12 electrodes (8 mm interelectrode distance) conductance micromanometer tipped catheter (CD Leycom, Zoetermeer, The Netherlands) was inserted through the right ventricular (RV) infundibulum into the right ventricle and positioned so that all electrodes were in the RV cavity. A 6F Fogarty balloon catheter (Baxter Healthcare Corp., Oakland, CA) was advanced into the inferior vena cava through a right femoral venotomy. Inflation of this balloon produced a gradual preload reduction.

Experimental protocol

A sample of venous blood was collected before baseline measurements and was allowed to clot in sampling tubes for 90 minutes, then cut into 3- to 5-mm cubes. After a 30 min stabilisation period, baseline measurements (Bas) were recorded. Thereafter, 12 animals were randomly divided in two groups. The first group (BM group, n = 6), received continuous infusion of BM-573 until the end of the experiment. BM-573 was synthesised in the laboratory of Medicinal Chemistry of the University of Liege as previously described (Rolin et al., 2001). It was dissolved in propylene glycol and water (10 % v/v) to achieve a drug solution of 20 mg/ml. After sterile filtration, the solution was administrated intravenously (10 mg/kg/h) leading to a steady state. This dosage has been chosen according to previous pharmacokinetic studies realized with BM-573 (Rolin et al., 2003; Lambermont et al., 2004; Dogne et al., 2004). The second group (Placebo group, n = 6) was perfused with equivalent volume of the same vehicle but without BM-573. After 30

minutes of pre-treatment, measurements were repeated (T0), then embolization of 0,3 g/kg of clots was carried out slowly through the external jugular vein for 5 to 10 minutes.

Besides, to assess the independent effects of BM-573, we measured hemodynamic parameters in pigs without embolism, before and after BM-573 infusion (BM-wpe group, n = 5) and in sham-operated control animals (Sham group, n = 5).

Data collection

Hemodynamic data included heart rate, systemic arterial pressure, pulmonary artery pressure (PAP) wave, pulmonary blood flow wave, left atrial pressure, RV pressure and volume. RV pressure-volume loops were monitored online throughout the experiment, recorded every 30 minutes from baseline to T240 during a short apnoeic phase, and stored for subsequent analysis. All analog signals were continuously converted to digital form with an appropriate system (Cudas, DataQ instruments inc., Akron, OH, USA). Left atrial pressure was maintained stable throughout the experiment by Ringer-lactate infusion as needed. RV pressure-volume loops were also recorded every 30 minutes from baseline to T240 during a transient occlusion of the inferior vena cava using the Fogarty balloon. The pressure and flow waves were sampled at 200 Hz and stored on files. Cardiac cycles were delimited by R wave detection provided by a permanent recording of a one lead electrocardiogram. Ten consecutive cycles were recorded during apnoea and numerically averaged to obtain representative diagrams of pressure and flow waves corresponding to specific experimental conditions.

Data analysis

Pulmonary circulation

Arterial elastance (E_a), which reflects RV afterload, was calculated using the following equation (Fourie et al., 1992):

$$E_a = (R_1 + R_2) / [T_s + R_2 C (1 - e^{-R_2 C / T_d})]$$

where T_s and T_d are the systolic and diastolic time intervals, respectively.

A four-element windkessel model, was used to assess the changes of the pulmonary vascular bed properties throughout the experimental protocol. In this model, a resistor (R_2) represents the resistive properties of the pulmonary vasculature, which are considered to reside primarily in the arteriolar system. A capacitor (C), is placed in parallel with R_2 and represents the compliant properties of the pulmonary arterial tree. A second resistor (R_1) is added at the input end of the circuit and reflects the characteristic impedance of the proximal pulmonary stem and its value depends on the calliper and compliance of the main pulmonary artery. Finally, an inductance (L) is added in series to allow positive phases angles between flow and pressure waves and accounts for the inertial properties of the blood and for the viscous resistive properties of the vessels wall (Grant and Paradowski, 1987; Lambermont et al., 1998). The relationship between pressure and flow in standard models is described by a second order linear differential equation (Lambermont et al., 1998):

$$a_0 Q + a_1 dQ/dt + a_2 d^2Q/d^2t = b_0 P + b_1 dP/dt + b_2 d^2P/d^2t \text{ (Eq.1)}$$

In order to avoid the use of a second derivative, which decreases signal-to noise ratio, Eq.1 is integrated and becomes:

$$\int_{t_0}^t Q dt = k_1 \int_{t_0}^t P dt + k_2 (P(t) - P(t_0)) + k_3 (Q(t) - Q(t_0)) + k_4 (dQ/dt - dQ/dt(t_0))$$

(Eq. 2)

where: Q = pulmonary flow, P = pulmonary pressure, t_0 = the beginning of the systole defined as the R wave on the ECG.

k_1 , k_2 , k_3 and k_4 were the following respective functions of L , R_1 , C and R_2 :

$$k_1 = \frac{1}{R_1 + R_2} \quad k_2 = \frac{C R_2}{R_1 + R_2} \quad k_3 = -\frac{L + C R_1 R_2}{R_1 + R_2} \quad k_4 = -\frac{L C R_2}{R_1 + R_2} \quad (\text{Eq.3})$$

The values of the hemodynamic parameters L , R_1 , R_2 and C were then derived by solving Eq.3.

Right ventricular function

RV pressure-volume loops were obtained using the conductance catheter method as previously described (Dickstein et al., 1995). Briefly, a multiple-electrode catheter placed in the right ventricle is used to set up an electrical field, and adjacent pairs of electrodes measure the local conductivity of blood, which is proportional to local blood volume (Dickstein et al., 1995). Structures surrounding the blood-filled ventricular cavity also contribute to the overall conductance signal. The resulting offset, termed parallel conductance, can be estimated by transiently altering the conductivity of blood with hypertonic saline (Dickstein et al., 1995). In addition, the conductance signal must be corrected to represent absolute volume. Therefore, to determine the gain factor (α slope factor), an alternate method of measuring volume is needed (Dickstein et al., 1995). In this study, we used the value of stroke volume measured by the pulmonary artery ultrasonic flow probe. Before each measurement, parallel conductance was determined with the saline method by injecting 3 ml of NaCl 10% into the inferior vena cava (Dickstein et al., 1995). During a rapid inferior vena cava occlusion maneuver, end-systolic elastance (E_{es}) was determined (Dickstein et al., 1995). End-systolic pressure-volume relationship was determined by fitting a straight line through the end-systolic pressure-volume points. In the time-varying elastance model of the ventricle, the total energy generated by each contraction is represented by the total area under the end-systolic pressure-volume relation line and the systolic segment of the pressure-volume trajectory and above

the end-diastolic pressure-volume relation curve. This area serves as a reliable predictor of myocardial oxygen consumption and was designed as pressure-volume area (PVA) (Fourie et al., 1992). Besides, the area within a pressure-volume trajectory loop represents external mechanical stroke work (SW). In fact, PVA consists of SW performed during systole and elastic potential energy (PE) presumed to be restored in the myocardium at end systole. Efficiency refers to energy conversion, and is defined as the ratio between useful energy, represented by mechanical SW to the energy supply to it, represented by PVA (Burkhoff and Sagawa, 1986; Fourie et al., 1992). Additionally, to assess right ventricular-vascular coupling, we examined the E_{es}/E_a ratio. Under normal operating conditions, the right ventricle operates at a maximum efficiency and at submaximal stroke work ($E_{es}/E_a > 1$). The maximal stroke work is obtained when $E_{es}/E_a = 1$, while uncoupling occurs when E_{es}/E_a is lower than 1 (Burkhoff and Sagawa, 1986; Fourie et al., 1992; Kass and Kelly, 1992).

Measurements of 6-keto-PGF_{1α} and TXB₂ plasma levels

Blood samples were collected using tubes containing 1:9 citrate (final conc. 0.38%). Platelet poor plasma was obtained by recentrifugation of the supernatant at 1200×g for 10 min. The production of TXA₂ metabolite, thromboxane B₂ (TX B₂), and of prostacyclin metabolite (6-keto-PGF_{1α}), was measured by using two competitive enzyme immunoassay kits (TXB₂ EIA kit and 6-keto-PGF_{1α} EIA kit, Cayman Chemical Company, Ann Harbor, CA, USA) according to previously described method (Rolin et al., 2001). Blood was sampled at baseline and T0, then every hour until T240.

Ex vivo platelet aggregation study

The antiplatelet potency of BM-573 was determined according to a previously described method (Dogne et al., 2000b). Briefly, blood samples were collected using tubes containing 1:9 citrate (final conc. 0.38%). The platelet-rich plasma (PRP) was obtained from the supernatant fraction after centrifugation for 20 min at 90 g (25°C). The remaining blood was centrifuged at 1200 g for 10 min (25°C) and the supernatant gave the platelet-poor plasma (PPP). The platelet concentration of PRP was adjusted to 3.10^8 cells.mL⁻¹ by dilution with PPP. Aggregation tests were performed according to Born's turbidimetric method by means of four-channel aggregometer (bioData Corporation, PAP4) (Born and Cross, 1963). PPP was used to adjust the photometric measurement to the minimum optical density. PRP (225 µL) was added in a silanized cuvette and stirred (1100 rev.min⁻¹). Platelet aggregation was initiated by addition of (5 µL) arachidonic acid (600 µM final) or (1 µL) U-46619 (1 µM final). To evaluate platelet aggregation, the maximum increase in light transmission (platelet aggregation amplitude) was determined from the aggregation curve 6 min after addition of the inducer.

Statistical analysis

Global between-group and within-group evolutions of response variables were investigated by means of linear mixed models. For a response Y_{ijk} measured at time t_i on pig j from group k , the model is given by:

$$Y_{ijk} = \alpha_k + \beta_k t_i + \gamma_k t_i^2 + b_{j0} + b_{j1} t_i + b_{j2} t_i^2 + \varepsilon_{ijk}$$

where α_k , β_k and γ_k are the group-specific fixed effects for the mean structure, b_{j0} , b_{j1} and b_{j2} the subject-specific random effects, and ε_{ijk} the measurement error term. Estimation and inference about both fixed and random effects were performed using the SAS software (SAS Institute Inc., Cary, NC, USA). Approximate F-tests (at 5 %

significance level) were used, first to look at potential differences in mean responses evolutions between the groups (by appropriate hypothesis testing on fixed effect parameters α_k , β_k and γ_k), and next for determining within-group evolution of the responses along the study time period (by testing statistical significance of time parameters). Therefore, p-value < 0.05 refers to either an established difference between groups in mean evolution of parameter values, or a non-constant time evolution of parameter values within one of the two groups of pigs. Longitudinal data analysis, although drawn on the whole time period under study, remains valid for any restricted time window. For practical purpose, a longitudinal data analysis restricted to the T0 to T30 period of time was added in the mean PAP evolution study.

Evolution of TXB₂ and 6-keto-PGF_{1 α} plasma levels and platelet aggregation amplitude were studied at each observation time by means of the Wilcoxon (Mann-Whitney) test, allowing comparison of population means without assuming a specific distribution for the variable. Reported p-values indicate whether the true between-group mean difference is significantly different from zero.

Results

Hemodynamic effects of blood clots embolization (Placebo group)

Analysis of within group parameters evolution in the Placebo group revealed that blood clots injection was followed by a significant ($p < 0.001$) increase in heart rate, while mean blood flow and systemic blood pressure were maintained. Mean PAP evolution showed a rapid initial increase ($p < 0.001$), followed by stabilisation above 30 mmHg throughout the experiment (Fig. 1).

As depicted in figure 2, windkessel model analysis pointed out a concomitant increase in R_2 and a decrease in C ($p < 0.001$), while R_1 remained unchanged. As a consequence, the increase in E_a was significant ($p < 0.001$) (Fig. 2).

Figure 3 illustrates the global evolution of end-diastolic volume and E_{es} . Both parameters significantly increased throughout the experiment ($p < 0.001$ and $p = 0.0025$, respectively). However, the increase in E_{es} was insufficient, because of such an augmented E_a , to maintain coupling values at their initial levels. Consequently, E_{es}/E_a ratio was significantly reduced ($p < 0.001$). It also achieved values associated with a significant ($p < 0.001$) reduction in right ventricular mechanical efficiency (Fig. 4).

Comparison between Placebo and BM groups

Comparison between groups showed no significant inter-group differences in heart rate, mean systemic arterial pressure, mean pulmonary flow, R_1 and C courses (Fig. 1-2). In contrast, as compared with the Placebo group, initial increase in mean PAP and R_2 were significantly reduced in the BM group ($p = 0.0171$ and $p = 0.025$ respectively), leading to significantly lower E_a values ($p = 0.007$) (Fig. 1-2). It should be noticed that BM-573 infusion induced a slight reduction of mean PAP between baseline and T0. However, further between group evolution was not explained by

this minor effect. Indeed, mean PAP increase between T0 and T30 was significantly higher in the Placebo group ($p=0.02$).

Between groups comparison of end-diastolic volume evolution revealed a trend towards lesser increase in the BM group ($p = 0.05$), while E_{es} also increased to statistically lower levels ($p = 0.039$) (Fig. 3). Such an evolution of E_{es} and E_a led to statistically higher coupling values ($p = 0.037$) in the BM group (Fig. 4). Finally, inter-group analysis revealed statistically different evolution in efficiency which remained higher in the BM group ($p = 0.001$) (Fig. 4).

Effects of BM-573 on hemodynamic parameters in pigs without embolism: comparison between the BM-wpe and Sham groups

To assess the independent effects of BM-573 on right ventricular hemodynamics, we measured the hemodynamic parameters in five pigs without embolism, before and every 30 minutes after BM-573 infusion until 240 minutes, and in five sham-operated control animals during the same time interval. In accordance with previous studies by our group (Lambermont et al., 2004; Rolin et al., 2003), comparison of time course evolution revealed no effect of BM-573 on mean blood flow, heart rate, aortic blood pressure, R1, R2, L, E_a , end-diastolic volume, E_{es} , E_{es}/E_a ratio and efficiency. The only difference noticed was that the increase in mean PAP with time was slightly greater in the sham-operated control animals (from 10.8 ± 1.4 mmHg to 15.5 ± 2.1 mmHg) than in the group receiving BM-573 (from 14.33 ± 1.03 mmHg to 16.49 ± 0.9 mmHg). This mild effect was secondary to a decrease in C (from 4.43 ± 0.33 to 2.99 ± 0.97 ml/mmHg) in the sham-operated control group only.

Effect of BM-573 on platelet aggregation induced by arachidonic acid and U-46619

Platelet aggregation amplitude was evaluated in BM and Placebo groups. Thirty minutes before BM-573 infusion, ex vivo platelet aggregation induced by arachidonic

acid or U-46619 was complete and irreversible. Intravenous injection (10 mg/kg/h) of BM-573 resulted in a complete inhibition of platelet aggregation provoked by arachidonic acid at T0, T120, T180 and T240 (Fig 5). The same antiplatelet effect was observed when U-46619 was used as inducer. In the Placebo group, platelet aggregation induced by both inducers remained complete and irreversible throughout the experiment.

Plasma levels of TXB₂ and 6-keto-PGF_{1α}

In the Placebo group, plasma concentration of TXB₂ and 6-keto-PGF_{1α} revealed an early massive release of TXB₂, while 6-keto-PGF_{1α} increased linearly (Fig. 6). BM-573 completely blunted TXB₂ secretion following pulmonary embolism ($p < 0.01$ at each observation time), but preserved 6-keto-PGF_{1α} liberation which increased similarly in both groups.

Gas exchange parameters

In the Placebo group, gas exchange parameters values revealed that blood clots injection caused a significant decrease in pH value and in arterial oxygen tension to inspired oxygen fraction ratio ($\text{PaO}_2/\text{FiO}_2$) ($p < 0.001$), whereas arterial carbon dioxide tension (PaCO_2) levels raised significantly ($p = 0.0006$) (Table 1).

Inter-group comparison revealed a significant difference towards lower levels in pH in Placebo group ($p = 0.0354$) with a non significant trend ($p = 0.058$) towards higher PaCO_2 levels. There was no significant inter-group difference in the course of $\text{PaO}_2/\text{FiO}_2$.

Discussion

Acute embolic pulmonary hypertension not only arises from direct mechanical obstruction of vessels by blood clots but also in part from active pulmonary vasoconstriction (Smulders, 2000). This latter accounts for multiple attempts at reversing induced pulmonary vasoconstriction. In the present study, we focused on the evaluation of the effects of BM-573 in an experimental model of acute pulmonary embolism. Our results evidence that BM-573 reduced the vascular load opposed to the right ventricular ejection during embolic obstruction.

Blood clots injection constantly induced a sharp increase in E_a , resulting from a prominent rise in R_2 , an increase in heart rate and a decrease in C , whereas R_1 , which reflects the resistive property of the main pulmonary artery was not modified by insult. BM-573 infusion reduced significantly such an afterload increase due to a preponderant effect on R_2 , while heart rate and C remained unaffected by the drug infusion.

Right ventricular contractility was clearly increased after embolization in both conditions, as evidenced by the significant E_{es} augmentation. In the BM group, such an homeometric adjustment was sufficient to regulate RV output. In contrast, in the Placebo group further regulation by the Frank-Starling mechanism was necessary to maintain cardiac output. In other words, increments in E_a was coupled with preload recruitment to maintain RV performance. This feature is in agreement with previous studies suggesting the intervention of these two adaptative mechanisms in case of RV outflow obstruction (de Vroomen et al., 2000; Hon et al., 2001; Lopes Cardozo et al., 2000; Rose, Jr. et al., 1983).

Animals pretreated with BM-573 experienced no significant alteration in RV mechanical efficiency. In the Placebo group, mechanical efficiency was significantly reduced and associated with a shift towards optimal coupling values, which contrast with hemodynamics findings during baseline where right ventricle operated at maximum efficiency rather than optimal coupling. Our data evidence that in response to afterload increases, E_{es} rise up to a point of optimal coupling but submaximal efficiency. BM-573 infusion prevented this deleterious effect in such a way that right ventricular-vascular coupling was maintained at maximal efficiency level. In contrast, right ventricle turned to a pressure pump operating at maximal coupling and minimal efficiency in the Placebo group.

Cyclooxygenase inhibition impairs not only TXA_2 synthesis but also prostaglandins production. This lack of selectivity may explain the apparent inconsistent effects of such an inhibition in the sub-acute phase of pulmonary embolism (Delcroix et al., 1992; Smulders, 2000). Several attempts to antagonise TXA_2 -mediated embolic effects were conducted through the use of selective TXA_2 synthase inhibitors like imidazole derivatives dazoxiben (UK 37248) (Garcia Szabo et al., 1983) and ozagrel (OKY 046) (Fukushima and Kobayashi, 1986; Ishihara et al., 1986; Lelcuk et al., 1987), which prevent the conversion of PGH_2 to TXA_2 . The main advantage offered by these compounds is to preserve prostacyclin production. Results were however disappointing, revealing either transient or only mild effects on hemodynamics (Fukushima and Kobayashi, 1986; Garcia-Szabo et al., 1988; Ishihara et al., 1986; Lelcuk et al., 1987; Utsonomiya et al., 1982). These results were explained by the incomplete TXA_2 synthase blockade and the accumulation of PGH_2 , a TXA_2 precursor, acting at common receptors (FitzGerald, 1991; Rolin et al., 2001). Others compared the effects of inhibition of TXA_2 synthase (with dazoxiben) with antagonism

of the TXA₂/PGH₂ receptor (with L-640,035) in a model of thrombin-induced pulmonary microembolism (Garcia-Szabo et al., 1988). These authors evidenced a protective effect on the progressive elevation of pulmonary vascular resistance.

The potential pharmacological interest of BM-573 is based on both inhibition and blockade of TXA₂ receptor. Regarding TXA₂ receptor antagonism, we evidenced in a previous study performed in anesthetized pigs, that BM-573 dose-dependently blocked pulmonary hypertensive effects induced by intravenous injection of the stable TXA₂ agonist U-46619 (Lambermont et al., 2003). These data were in agreement with the study of Rolin et al. who showed in vitro that BM-573 was a strong smooth muscle TXA₂ receptor antagonist (Rolin et al., 2001). The present study evidenced that infusion of BM-573 resulted in a complete prevention of ex vivo platelet aggregation induced by arachidonic acid, the TXA₂ precursor and U-46619, the stable TXA₂ agonist. These results confirm the efficacy of BM-573 as a potent TXA₂ receptor antagonist (Rolin et al., 2003; Dogne et al., 2004).

Regarding the TXA₂ / prostacyclin balance, our results demonstrate that BM-573 blocked the TXA₂ synthesis but preserved the prostacyclin liberation. This is in agreement with previous results concluding that BM-573 does not exhibit a cyclooxygenase inhibition (Dogne et al., 2002). In our experimental model of acute pulmonary embolism, plasma concentration of TXB₂ and 6-keto-PGF_{1α}, the stable metabolites of thromboxane and prostacyclin respectively, confirmed that massive release of TXA₂ preceded prostacyclin response. Biosynthesis of prostacyclin and TXA₂ are elevated in human syndrome of platelet activation (Oates et al., 1988) and, as a natural antagonist, endogenous prostacyclin appears to modulate the cardiovascular action of TXA₂ (Cheng et al., 2002). This may be highly relevant in pulmonary embolism because prostacyclin release reaches its peak level after TXA₂,

with therefore a transient situation of increased TXA₂ without simultaneous prostacyclin augmentation. Pulmonary vasoconstriction may be most important in early steps and antagonists of vasoconstrictive mediators may be more effective when used in the initial phase of hemodynamic instability whereas blocking prostacyclin synthesis or action would result in further hemodynamic deterioration (Smulders, 2000).

Hypoxemia induced by blood clots embolization was however not prevented by BM-573 pretreatment. Similar results have been found with endothelin-1 antagonist ZD2574. Endothelin-1 is known to be an activator of the cyclooxygenase pathway, resulting in enhanced TXA₂ formation (Lee et al., 2001). In acute canine autologous blood clot pulmonary embolism, Delcroix *et al.* demonstrated that pharmacologic reduction in pulmonary vascular tone by hydralazine and nitroprusside had no effect on gas exchange and ventilation to perfusion distribution, although pulmonary hypertension was reduced (Delcroix et al., 1990). On the contrary, in the same conditions, cyclooxygenase inhibition was shown to deteriorate gas exchange probably because of an inhibited production of bronchodilating prostaglandins or recruitment of previously unperfused embolized areas due to increased pulmonary artery pressure (Delcroix et al., 1992).

Several limitations of this study must be noted. First, the pharmacological effects of BM-573 were not specifically compared with a selective TXA₂ synthase inhibitor, receptor antagonist or cyclooxygenase inhibitor. BM-573 is a potent TP receptors antagonist, able to prevent not only TXA₂ but also other mediators such as prostaglandin D₂, 8-iso-PGF_{2α} and endoperoxide prostaglandin H₂ from activating these receptors. Thus, the TXA₂ synthase inhibition property of BM-573 may not be necessary for right heart overload protective effect. Besides, TXA₂ synthase inhibition

blunts TXA₂ overproduction but maintains prostacyclin liberation, which may, in turn, acts as a vasodilating and antiaggregating agent by activating its own receptors (IP). As a result, the effect of pulmonary embolism observed in the BM group may be dependent on that prostacyclin liberation as well as on the antagonism of the TP receptors. Thus, dual activity could be unnecessary for BM-573 preventive effect and further experiments should focus on the evaluation of the specific contributions of TXA₂ receptor antagonism and TXA₂ synthase inhibition in pulmonary embolism.

Secondly, clinical extrapolation of the present findings made under conditions of animal experimentation should be made with caution. Indeed, our experimental preparation involved use of fresh clots cut in small pieces with a large surface area leading to more platelet activation compared to what occurs in human clinical setting. It is therefore likely that patients suffer quantitatively less from humoral mediated effects than those noted in our study. Only the very acute effects of blood clot embolization were investigated, which does not allow to forecast subsequent right ventricular-vascular adaptation. Finally, BM-573 was used as a pretreatment in order to inhibit the very early TXA₂ secretion. Therefore, present results are restricted to the field of preventive therapy.

In conclusion, this study evidenced that pretreatment with BM-573 limited the early hemodynamic alterations secondary to pulmonary embolism by reducing pulmonary vasoconstriction. Consequently, ventricular-vascular coupling was maintained at maximal efficiency level.

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Footnotes

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Legends for figures:

Figure 1: Time course of conventional hemodynamic parameters in Placebo group (open square) and in BM group (closed square). Data are presented as mean \pm standard error of the mean. *** indicates significant ($p < 0.001$) change of parameter value over time in Placebo group. \$\$\$ indicates significant ($p < 0.001$) change of parameter value over time in BM group. † indicates significant ($p < 0.05$) between group difference in mean evolution of parameter value.

Figure 2: Time course of pulmonary characteristic resistance (R_1), distal resistance (R_2), total pulmonary compliance (C) and arterial elastance (E_a) in the Placebo group (open square) and BM group (closed square). Data are presented as mean \pm standard error of the mean.

*** indicates significant ($p < 0.001$) change of parameter value over time in Placebo group. \$\$ and \$\$\$ indicate significant ($p < 0.01$ and $p < 0.001$, respectively) change of parameter value over time in BM group. † and †† indicate significant ($p < 0.05$ and $p < 0.01$, respectively) between group difference in mean evolution of parameter value.

Figure 3: Time course of right ventricular end-diastolic volume and end-systolic elastance (E_{es}) in the Placebo group (open square) and BM group (closed square). Data are presented as mean \pm standard error of the mean. ** and *** indicate significant ($p < 0.01$ and $p < 0.001$, respectively) change of parameter value over time in Placebo group. \$\$ indicates significant ($p < 0.01$) change of parameter value over time in BM group. † indicates significant ($p < 0.05$) between group difference in mean evolution of parameter value.

Figure 4: Time course of right ventricular-vascular coupling (E_{es}/E_a) and mechanical efficiency in the Placebo group (open square) and BM group (closed square). Data are presented as mean \pm standard error of the mean. *** indicates significant ($p < 0.001$) change of parameter value over time in Placebo group. § indicates significant ($p < 0.05$) change of parameter value over time in BM group. † and †† indicate significant ($p < 0.05$ and $p < 0.01$, respectively) between group difference in mean evolution of parameter value.

Figure 5: Time course of platelet aggregation amplitude induced by arachidonic acid in the Placebo group (empty columns) and BM group (black columns). Data are presented as mean \pm standard error of the mean. *** indicates significant ($p < 0.001$) between group difference at that observation time (Wilcoxon test).

Figure 6: Time course of TXB_2 and 6-keto $PGF_{1\alpha}$ plasma levels in the Placebo group (empty columns) and BM group (black columns). Data are presented as mean \pm standard error of the mean. ** indicates significant ($p < 0.01$) between group difference at that observation time (Wilcoxon test).

Gas exchange response after embolization

Parameter	Group	Time						
		Baseline	T0	T60	T120	T180	T240	
pH	Placebo	7.53±0.02	7.50±0.02	7.33±0.03	7.31±0.05	7.31±0.05	7.29±0.06	***
	BM	7.52±0.01	7.53±0.01	7.39±0.03	7.36±0.04	7.34±0.04	7.33±0.05	\$\$\$ †
PaO ₂ /F _i O ₂ , mmHg	Placebo	531±37	505±30	235±52	207±31	232±45	196±51	***
	BM	459±38	467±44	319±69	289±51	213±35	242±32	\$\$\$
PaCO ₂ , mmHg	Placebo	33.2±0.8	35.2±0.8	56.8±4.9	58.5±6.1	58.7±5.8	64±8.5	***
	BM	33.6±0.4	32.5±1.5	49.1±3.5	47.1±2.9	51.6±3.9	51.6±4.1	\$\$\$

Table 1. Data are presented as means ± SEM. *** indicates significant (p<0.001) change of parameter value over time in Placebo group. \$\$\$ indicates significant (p<0.001) change of parameter value over time in BM group. † indicates significant (p<0.05) between group difference in mean evolution of parameter value.

Figure 1

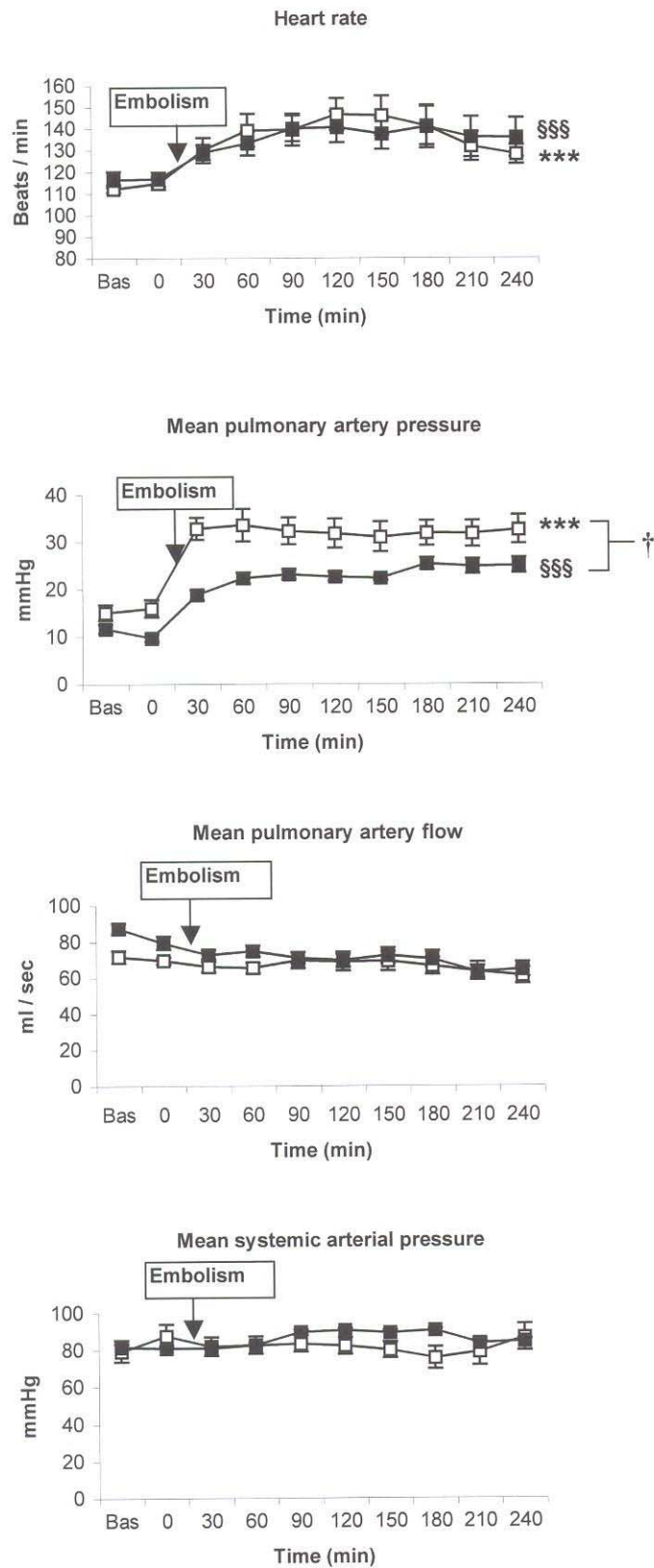


Figure 2

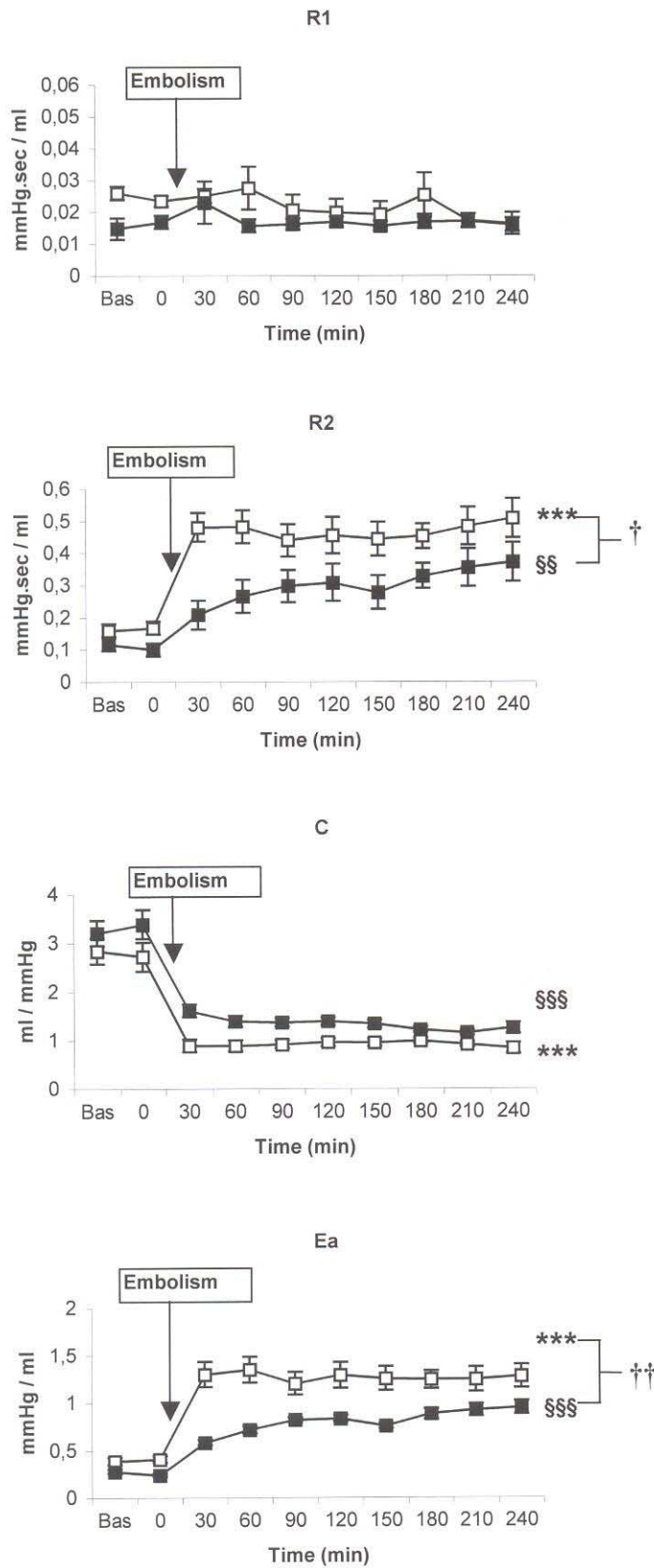


Figure 3

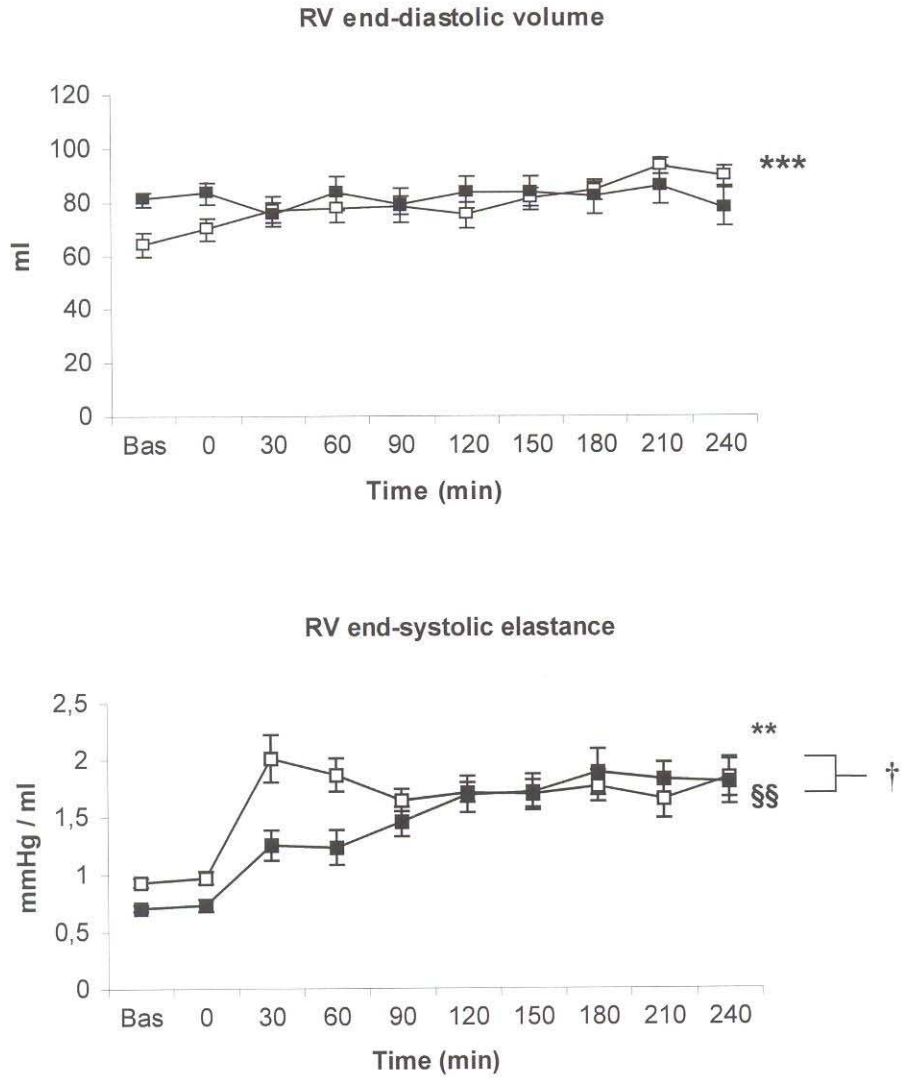


Figure 4

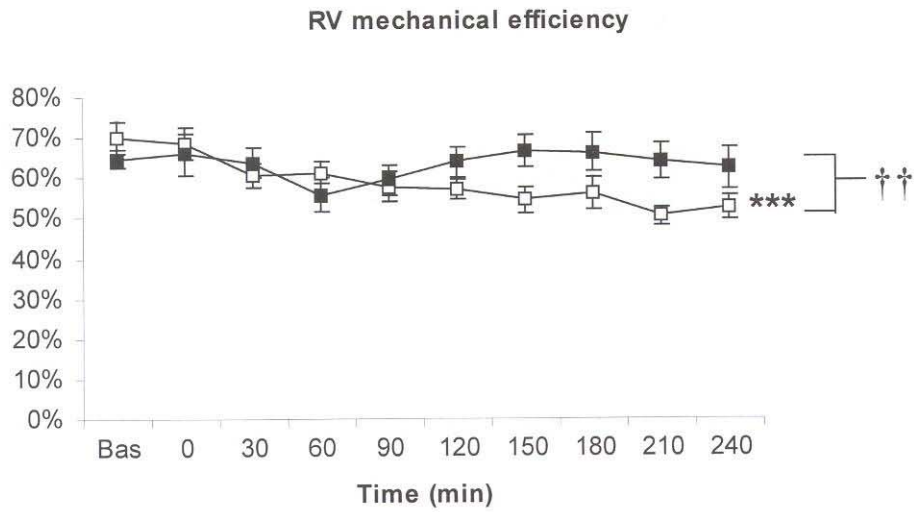
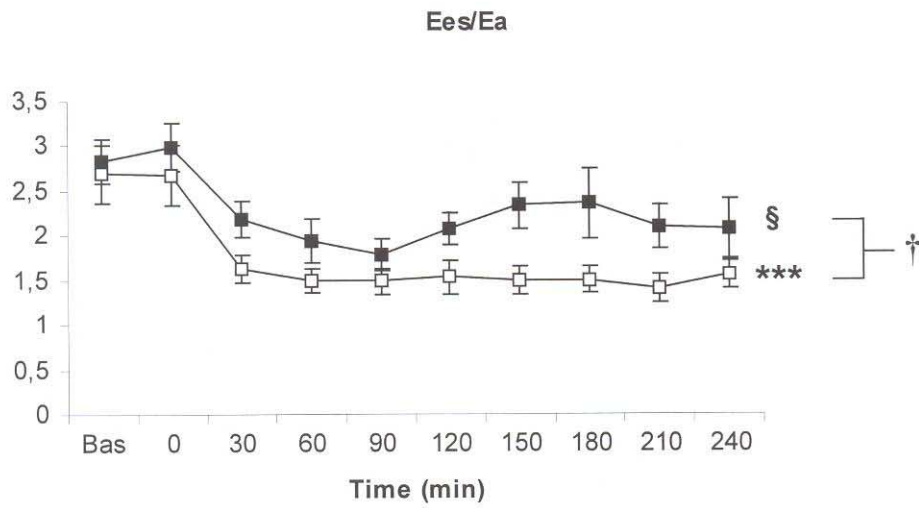


Figure 5

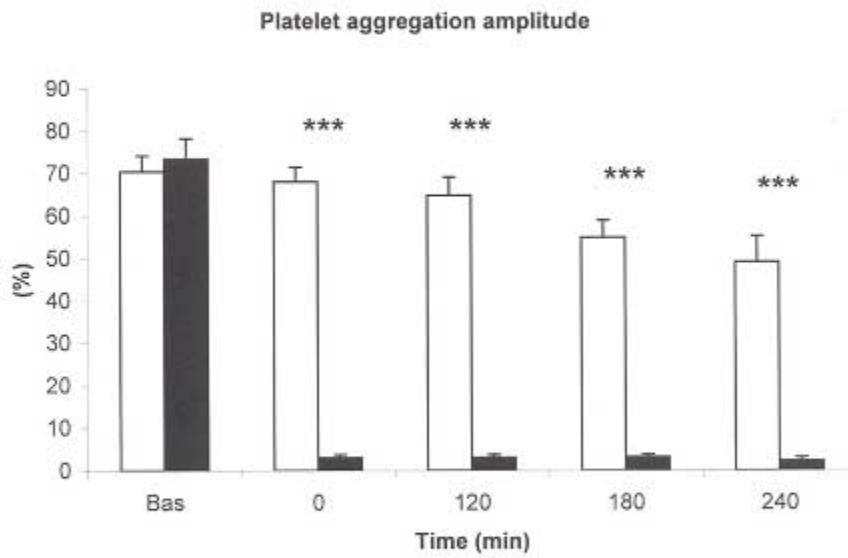


Figure 6

