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Effects of U-46619 on Pulmonary Hemodynamics Before and After Administration of BM-573, a Novel Thromboxane A_2 Inhibitor

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Abstract

We studied the effects on pulmonary hemodynamics of U-46619, a thromboxane A_2 (TXA_2) agonist, before and after administration of a novel TXA_2 receptor antagonist and synthase inhibitor (BM-573). Six anesthetized pigs (Ago group) received 6 consecutive injections of U-46619 at 30-min interval and were compared with six anesthetized pigs (Anta group) which received an increasing dosage regimen of BM-573 10 min before each U-46619 injection. Consecutive changes in pulmonary hemodynamics, including characteristic resistance, vascular compliance, and peripheral vascular resistance, were continuously assessed during the experimental protocol using a four-element Windkessel model. At 2 mg/kg, BM-573 completely blocked pulmonary hypertensive effects of U-46619 but pulmonary vascular compliance still decreased. This residual effect can probably be explained by a persistent increase in the tonus of the pulmonary vascular wall smooth muscles sufficient to decrease vascular compliance but not vessel lumen diameter. Such molecule could be a promising therapeutic approach in TXA_2 mediated pulmonary hypertension as it is the case in pulmonary embolism, hyperacute lung rejection and endotoxinic shock.

Keywords: Models, pulmonary circulation, swine, thromboxane, vascular compliance, vascular resistance.

Introduction

Thromboxane A_2 (TXA_2) is a potent vasoconstrictor and aggregating agent produced by platelets, leukocytes and endothelial cells (Hamberg et al., 1975; Oates et al., 1988). It is a very labile molecule, with a half-life of approximately 30 secs in aqueous media, and it is hydrolyzed to form a stable but inactive metabolite, thromboxane B_2 (TXB_2) (Oates et al., 1988).

TXA_2 has been implicated as a pro-inflammatory mediator during the pulmonary hypertensive phase of Gram-negative sepsis (Wise et al., 1981) and of hyperacute lung rejection (Collins et al., 2001; Ogletree & Brigham, 1982). It is also an agent responsible for pulmonary vasoconstriction in pulmonary embolism (Smulders, 2000) and a pathophysiological mediator in thrombosis, vasospasm, arrhythmias and progression of ischemic damage after coronary occlusion (Oates et al., 1988).

In such pathological conditions, potential benefits of blocking TXA_2 effects were investigated by testing inhibitors of TXA_2 biosynthesis. These inhibitors were rapidly found ineffective because of an incomplete blockage of thromboxane synthase at the dosage used and because TXA_2 synthase inhibition induces the accumulation of a TXA_2 precursor called endoperoxide H_2 (PGH_2) which is chemically more stable and exerts similar biological effects by acting at common receptors. However, specific inhibitors of TXA_2 synthase are also responsible for increasing generation of prostanoids such as prostaglandin I_2, which can be beneficial by attenuating platelet activation and decreasing vascular resistance. Therefore, efforts to modulate the actions of TXA_2 focused on agents able to block biosynthesis of TXA_2 and to antagonize both PGH_2 and TXA_2 at their common receptor (Oates et al., 1988; Vandeplassche et al., 1991; Vandeplassche et al., 1993).

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BM-573 is a molecule derived from the pyridine sulfonylurea torasemide, a loop diuretic. It is obtained by the replacement of the pyridine ring of torasemide with a nitrobenzene and the presence of a tert-butyl group on the distal nitrogen of the sulfonylurea function which improved the TXA_2 antagonism and revealed TXA_2 synthase inhibitory potency (Fig. 1) (Rolin et al., 2001). This novel TXA_2 receptor antagonist and synthase inhibitor has a powerful antiplatelet potency and was shown to relax the rat aorta artery precontracted with U-46619 (Rolin et al., 2001).

As current evidence suggests that TXA_2 plays a role in pathophysiologic processes in the lung, we tested whether this new dual TXA_2 synthase inhibitor and TXA_2 receptor antagonist was able to antagonise pulmonary hemodynamic effects of U-46619, a stable TXA_2 agonist. Therefore, we studied the effects of U-46619 on pulmonary hemodynamics after administration of BM-573 using a four-element windkessel model (Grant & Paradowski, 1987; Lambermont et al., 1999).

Materials 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 European Community Guidelines for the use of experimental animals.

Experiments were performed on 12 healthy pure pietran pigs of either sex weighing from 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 15 mL/kg at a respiratory rate of 20 breaths/min. End-tidal CO_2 measurements (Capnomac, Datex, Helsinki, Finland) were used to monitor the adequacy of ventilation. Respiratory settings were adjusted to maintain end-tidal CO_2 between 30 and 35 mmHg. Arterial oxygen saturation was monitored closely and maintained at >95% by adjusting the FiO_2 as necessary.

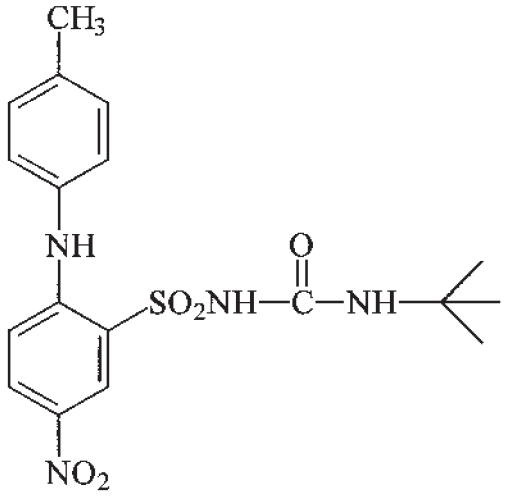

Fig. 1. Chemical structure of the TXA_2 receptor antagonist and synthase inhibitor BM-573.

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. Finally, systemic blood pressure was monitored with a micromanometer-tipped catheter inserted into the descending thoracic aorta through the left femoral artery.

Chemicals

U-46619 (Cayman Chemical, Ann Arbor, MI) supplied in ethanolic solution was diluted in NaCl 0.9% solution to a final concentration of 10 µg/ml. BM-573 was obtained from the laboratory of Medicinal Chemistry of the University of Liège. It was dissolved in propyleneglycol and water to achieve a drug solution of 20 mg/ml.

Experimental protocol

After a 30 min stabilization period, animals were randomisely divided in two groups. In the first group (n = 6) (Ago group) the animals received six consecutive injections of the same dose of U-46619 (1.25 µg/kg) at a 30 min interval. Hemodynamic data were recorded before each injection of U-46619 (TO) and 2 (T2), and 15 (T15) mins after each injection of U-46619. Hemodynamic data included pulmonary artery pressure wave (PAP), pulmonary blood flow wave (CO), left atrial pressure, systemic arterial pressure (BP), and heart rate (HR).

In the second group (n = 6) (Anta group), an increasing dosage regimen of BM-573 was administrated intavenously 10 min before each U-46619 injection, with the exception of the first U-46619 injection which was not preceded by an antagonist injection. The animals received 0.125 mg/kg of BM-573 before the second, 0.250 mg/kg before the third, 0.500 mg/kg before the fourth, 1 mg/kg before the fifth and 2 mg/kg before the sixth agonist injection. In the Anta group, hemodynamic data were recorded before each

antagonist injection (Anta 0, Anta 0.125, Anta 0.25, Anta 0.5, Anta 1, Anta2), 10 min later (i.e., just before agonist injection) (T0), and 2 (T2) and 15 (T15) min after each injection of U-46619.

In both groups, each sequence of agonist injection and the corresponding recordings of data (T0, T2, T15) were called Run 0, Run 0.125, Run 0.250, Run 0.500, Run 1, Run 2, respectively, according to the dose of antagonist injected in the Anta group.

Data collection

All analog signals were continuously converted to digital form with an appropriate system (Codas, DataQ instruments inc., Akron, OH). 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 apneic phase and numeri-cally averaged to obtain representative diagrams of pressure and flow waves corresponding to specific experimental conditions.

Data analysis

A lumped parameter model, namely the four-element windkessel model (WK4) (Fig. 2), was used to analyze the flow conditions in the pulmonary circulation throughout the experimental protocol. The resistor R_2 represents the resistive properties of the pulmonary vasculature which are considered to reside primarily in the arteriolar system. The capacitor C, placed in parallel with R_2 , represents the compliant properties of the pulmonary arterial tree (Grant & Paradowski, 1987). The resistor R_1 is used to reflect characteristic resistance which level depends prominently on the resistance and compliance of the main pulmonary artery. Finally, an inductance L is added 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 & Paradowski, 1987). The four elements of the model were simultaneously identified by using an original analytic procedure previously described (Lambermont et al., 1998).

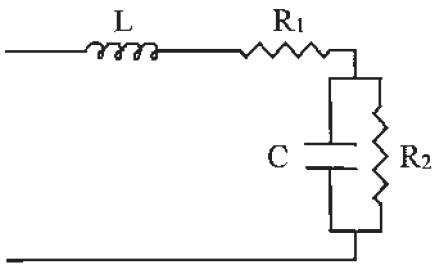

Fig. 2. Electrical representation of the four-element windkessel model. It is composed of a characteristic resistance (R_1), a peripheral resistance (R_2), a compliance (C), and an inductance (L).

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM).

A factorial design with 3 fixed effects (time, run, pig) of order 2 was used to determine the effects of time and run on the different parameters values (Statistica ’99 edition, Statsoft Inc, Tulsa, OK, USA).

For evaluation of differences between groups, Student’s *t*-test was applied.

$P < 0.05$ was considered statistically significant.

Results

Conventional hemodynamic variables

Time course of conventional hemodynamic variables for both groups is presented in Figure 3.

Ago group

In Ago group, PAP increased from a mean value of 22.8 ± 0.8 mmHg at T0 to 47.2 ± 1.7 mmHg ($p < 0.05$) 2 min after injection of U-46619 (T2) and returned to baseline values (25 ± 0.8 mmHg) 15 min later (T15). A progressive increase in PAP values at T0, T2 and T15 was observed between Runs (Fig. 3).

CO decreased from 91.3 ± 2.9 ml/s (T0) to 63 ± 4.2 ml/s (T2) ($p < 0.05$) after administration of U-46619. This decrease in CO following administration of U-46619 at T2 worsened over time. It decreased from 110 ± 2.8 to 96 ± 3.2 ml/s ($p < 0.05$) during the first Run (Run 0) and from 81 ± 3.1 to 47 ± 7.4 ml/s ($p < 0.05$) during the last Run (Run 2), while HR increased from 123 ± 3.4 beats/min (T0) to 137 ± 4.7 beats/min (T2) ($p < 0.05$) and returned to baseline values at T15 (122 ± 3.5 beats/min).

BP slightly increased following the injection of U-46619 in the first Runs (Run 0, Run 0.125, Run 0.250, Run 0.500, respectively) while in both Run 1 and Run 2 BP decreased slightly after U-46619 injection.

Anta group

There is no difference between Ago and Anta groups during Run 0. From Run 0.125, PAP showed an increase which was less important in Anta group than in Ago group ($p < 0.05$) while in Run 2, PAP remained constant after administration of U-46619. HR and CO were not influenced by the injection of U-46619 which followed a 0.125 mg/kg BM-573 injection (Run 0.125).

Pulmonary hemodynamics

Time course of calculated pulmonary vascular parameters as provided by the WK4 model is presented in Figure 4.

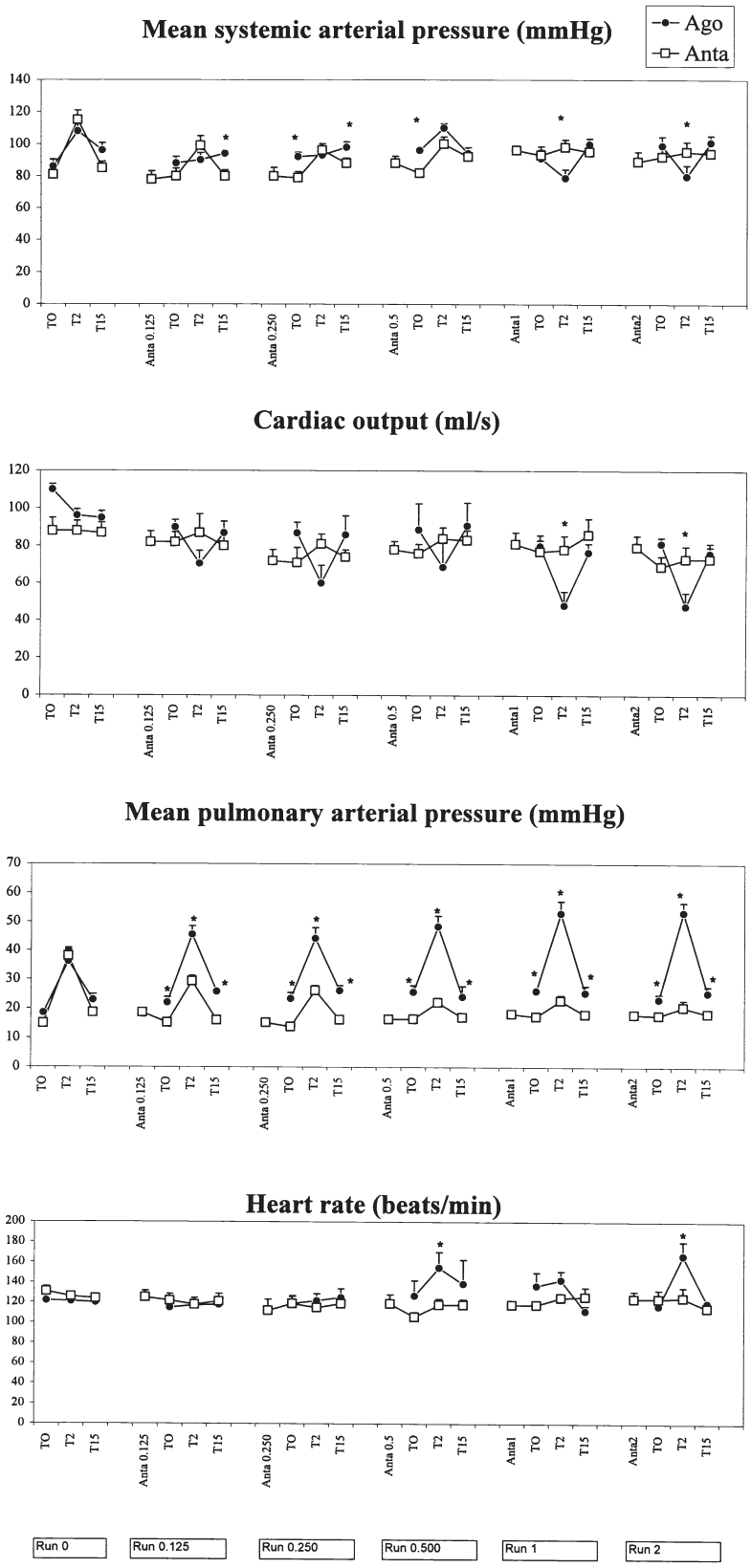

Fig. 3. Time course of conventional hemodynamic variables in the Ago group (Closed circle) and the Anta group (Open square) before U-46619 injection (T0), 2 min after U-46619 injection (T2) and 15 min after U-46619 injection (T15) in both Ago and Anta group and, in Anta group, before BM-573 injection (Anta 0.125, Anta 0.500, Anta 1, Anta 2, according to the dose of BM-573 injected (mg/kg)).

*$p < 0.05$ Ago group vs Anta group.

Ago group

R_1 and R_2 followed exactly the same pattern (Fig. 4). They increased at T2 and returned to baseline values at T15. Values at T0 were remarkably stable between Runs. C decreased largely at T2 during each Run and returned almost at the previous value of T0 with a slight decrease over time of T0 values.

Anta group

There was no difference between Ago and Anta groups during Run 0. From Run 0.125 and Run 0.250, R_1 and R_2, respectively, did not increase after administration of U-46619 and remained remarkably stable over time. However, U-46619 decreased C values at T2 in all Runs in both groups

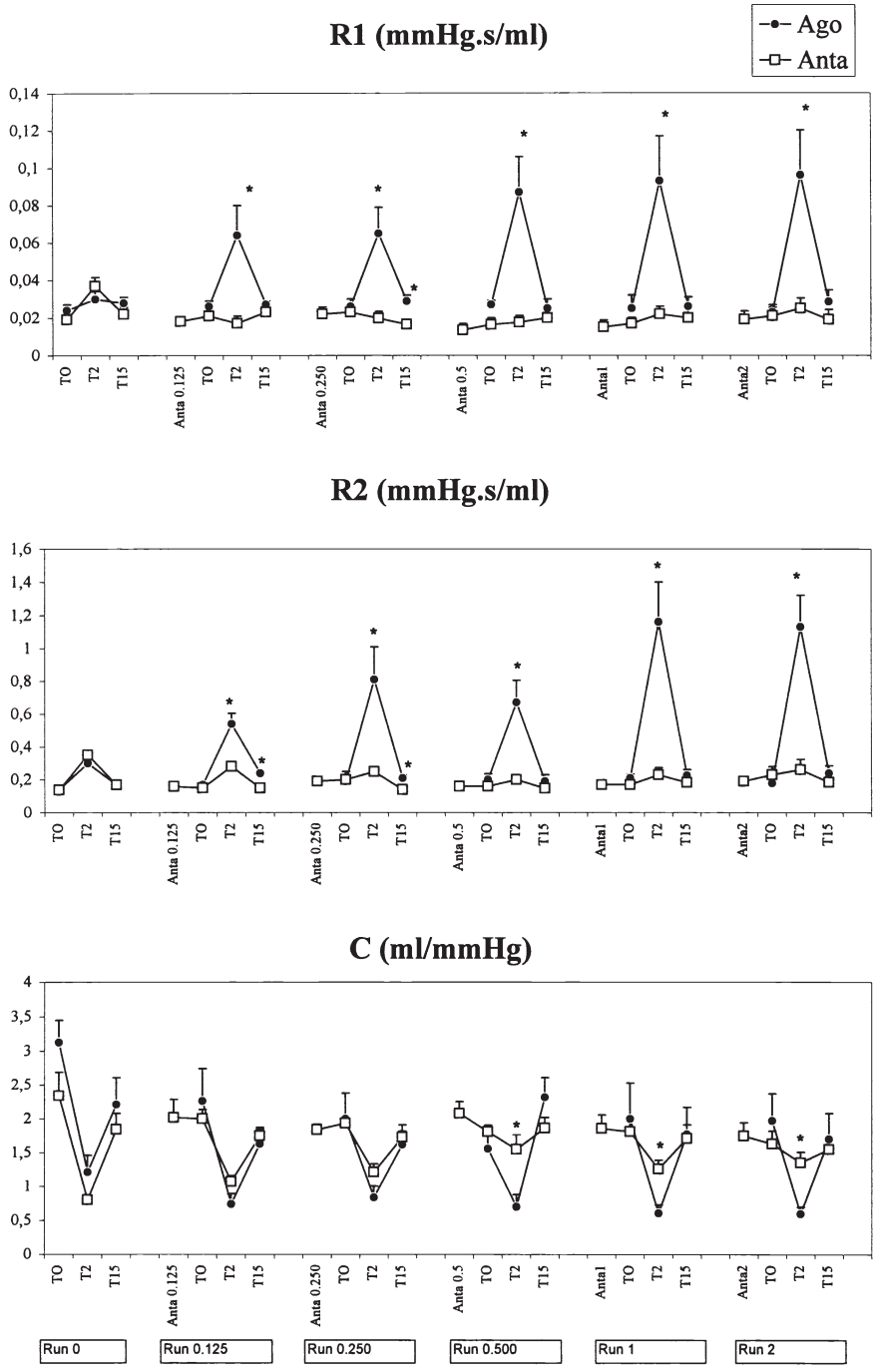

Fig. 4. Time course of calculated pulmonary vascular parameters as obtained by the four-element windkessel model of the pulmonary circulation in the Ago group (Closed circle) and the Anta group (Open square) before U-46619 injection (T0), 2 min after U-46619 injection (T2) and 15 min after U-46619 injection (T15) in both Ago and Anta group and, in Anta group, before BM-573 injection (Anta 0.125, Anta 0.500, Anta 1, Anta 2, according to the dose of BM-573 injected (mg/kg)). R_1, characteristic vascular resistance; R_2, peripheral vascular resistance; C, vascular compliance.

* $p < 0.05$ Ago group vs Anta group.

but C was significantly higher in Anta group than in Ago group at T2 from Run 0.5 ($p < 0.05$).

Discussion

Pulmonary vasonctriction caused by the release of predominantly vasoconstrictive mediators, is an important contributor to the increase in pulmonary vascular resistance in patients with acute pulmonary embolism, hyperacute lung rejection or during the early phase of septic shock (Collins et al., 2001; Lambermont et al., 1999; Smulders, 2000). In septic shock, right heart failure can lead to death (Kimchi et al., 1984) and, in pulmonary embolism, most patients who succumb do so within minutes to few hours after onset of symptoms. Therefore drugs that antagonise vasoconstrictive mediators could be effective overall if given in the initial phase of hemodynamic instability.

When pulmonary cyclooxygenase activity is inhibited, the importance of endothelial prostacyclin synthesis to preservation of transpulmonary blood flow is apparent. In contrast to selective antithromboxane strategy which results in preserved transpulmonary blood flow, inhibition of both prostacyclin and thromboxane in dogs with oleic acid lung injury is associated with significantly elevated pulmonary vascular resistance (Leeman et al., 1988). Wether TXA_2 directly affects pulmonary microvascular integrity is controversial. Nevertheless, TXA_2 has postvenular vasoconstrictive effects (Schuster et al., 2001) and therefore increases microvascular fluid transudation by causing increased hydrostatic pressure in pulmonary capillary beds (Collins et al., 2001).

TXA_2 has also proinflammatory effects and reduced TXA_2 effects generally correlates with a blunted inflammatory response (Collins et al., 2001).

In previous *in vitro* and *in vivo* studies, it has been demonstrated that BM-573 was a potent dual compound able to reduce TXA_2 production by thromboxane synthase inhibition and to prevent the action of TXA_2 (or PGH_2) by blocking the TXA_2 receptors (Rolin et al., 2001). The results of the present study evidence that the pulmonary vascular responses to the TXA_2 stable agonist, U-46619, were markedly reduced by a 2 mg/kg injection of BM-573.

As expected, in Ago group, U-46619 increased pulmonary artery pressure in response to increases in pulmonary vascular resistances (Kaye et al., 1995; Sjoberg & Steen, 1989). In Runs 1 and 2, PAP increased to a level high enough to provoke a fall in CO associated to a paradoxal decrease in BP.

Our data showed that BM-573 completely blocked pulmonary hypertensive effects of U-46619. However, at the regimen of 2 mg/kg and, in spite of the lack of effects on resistance, pulmonary vascular compliance still decreased after administration of the TXA_2 agonist. This residual effect can probably be explained by a persistent increase in the tonus of the pulmonary vascular wall smooth muscles sufficient to decrease vascular compliance but not vessel lumen diameter. The inhibition of TXA_2 synthase by BM-573 was not investigated in the present study but, as previously demonstrated, BM-573 completely reduced the platelet production of TXB_2 induced by arachidonic acid (Rolin et al., 2001).

In essence, our data suggest that the present antagonist could be a promising therapeutic approach in the field of bocking TXA_2 in pulmonary hypertension secondary to pulmonary embolism, hyperacute lung rejection or septic shock not only because BM-573 administration blocks TXA_2 induced pulmonary vasoconstriction but also because it can prevent platelet aggregation and decrease inflammatory response.

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