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Effects of BM-573, a thromboxane A_2 modulator on systemic hemodynamics perturbations induced by U-46619 in the pig

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Abstract

The aim of our study was to evaluate the effects of thromboxane A_2 (TXA_2) agonist, U-46619, on systemic circulatory parameters in the pigs before and after administration of a novel TXA_2 receptor antagonist and synthase inhibitor (BM-573). Twelve anesthetized pigs were randomly assigned in two groups: in Ago group (n = 6), the animals received six consecutive injections of U-46619 at 30 min interval, while in Anta group (n = 6) they received an increasing dosage regimen of BM-573 10 min before each U-46619 injection. The effects of each dose of BM-573 on ex vivo platelet aggregation induced by arachidonic acid, collagen or ADP were also evaluated. Vascular properties such as characteristic impedance, peripheral resistance, compliance, arterial elastance were estimated using a windkessel model. Intravenous injections of 0.500 mg/ml of BM-573 and higher doses resulted in a complete inhibition of platelet aggregation induced by arachidonic acid. In the same conditions, BM-573 completely blocked the increase of arterial elastance, and stabilized both mean aortic blood pressure and mean systemic blood flow. In conclusion, BM-573 could therefore be a promising therapeutic approach in pathophysiological states where TXA_2 plays a main role in the increase of vascular resistance like in pathologies such as systemic hypertension.

Keywords: Thromboxane A_2; Systemic hemodynamics; Platelet aggregation; Hypertension

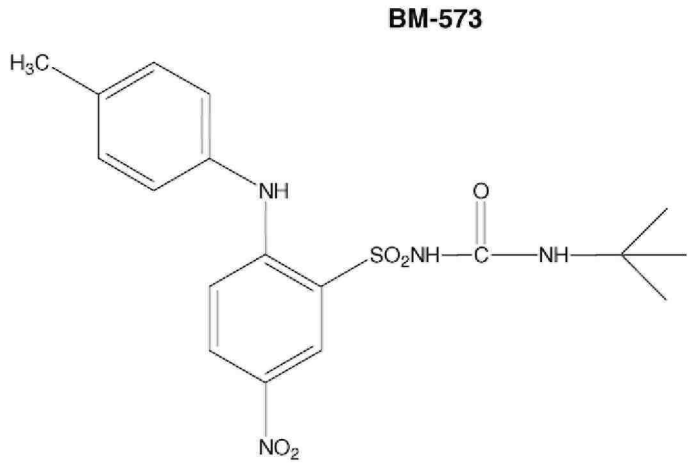
1. Introduction

Thromboxane A_2 (TXA_2), a labile metabolite of arachidonic acid generated through its sequential metabolism by cyclooxygenases and thromboxane synthase, is produced mainly by platelets, macrophages, vascular endothelial cells [1,2] but also by cardiomyocytes, suggesting some role in the heart [3,4]. TXA_2 induces platelet activation, and contraction of vascular and respiratory smooth muscles [5]. It has been implicated as a pathophysiological mediator in thrombosis, vasospasm, progression of ischemic damage after coronary artery occlusion and in occurrence of ischemia-reperfusion arrhythmias [6-9]. Furthermore, previous studies have suggested that TXA_2 contributes to vascular homeostasis through its actions as a potent vasoconstrictor and platelet aggregating agent [10-12]. Wilcox and collaborators also suggested a role for the TXA_2 receptors (TP) in the regulation of blood pressure and hemodynamic responses in various models of hypertension, vascular disease, and shock [13]. Moreover, TXA_2 has been implicated in the regulation of renal blood flow, sodium handling [11,14,15], and interaction with the rennin-angiotensin system [16,17]. Indeed, common actions of angiotensin II and TXA_2 may promote systemic and renal vasoconstriction, sodium handling, and vascular smooth muscle cell proliferation [18]. These interactions suggest a potential contribution of TXA_2 acting through the TP receptors in the pathogenesis of hypertension [19]. Moreover, I-Shing and collaborators in a study with TXA_2 synthase (TXAS) knockout mice showed that endoperoxides were also implicated in the control of blood pressure since TXAS knockout mice exhibited a paradoxical elevation of the mean arterial blood pressure following arachidonate infusion; this effect was prevented by the pretreatment of those mice with a selective TP antagonist [20].

BM-573 is a molecule derived from the pyridinic 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) [21]. 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, a stable TXA_2 agonist [21-23]. In the pig, our team demonstrated that BM-573 revealed a protective effect against pulmonary hypertension induced by U-46619 [24]. Besides, we also reported the preventive effect of BM-573 in the early phase of endotoxin induced pulmonary hypertension [25], and during embolic induced pulmonary vasoconstriction [26]. Finally, BM-573 also revealed a protective effect in a model of myocardial infarction induced by coronary thrombosis [22]. However, the effects of U-46619 on systemic hemodynamics remained to

be studied as well as the preventive role of BM-573 in such conditions. Therefore, in this study, we aimed to evaluate the effects of U-46619 on systemic circulatory parameters in the pig before and after administration of BM-573. Vascular parameters were assessed using a four-element windkessel model [25,27,28].

Fig. 1: *Chemical structure of the TXA2 receptor antagonist and synthase inhibitor BM-573.*



2. Materials and methods

2.1. Animal preparation

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 Liège. 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 25 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 sodium pentobarbital (3 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 above 95% by adjusting the FiO2 as necessary. Temperature was maintained at 37 °C by means of a heating blanket.

The chest was opened with a mid-sternotomy, the pericardium was incised and sutured to the chest wall to form a cradle for the heart, and the root of the aorta was dissected clear of adherent fat and connective tissue. A micromanometer-tipped catheter (Sentron pressure measuring catheter, *Cordis*, Miami, FL, USA) was inserted through the left carotid artery and advanced into the ascending aorta. A 14 mm diameter perivascular flow probe (Transonic Systems Inc., Ithaca, NY, USA) was closely adjusted around the aorta 2 cm distal to the aortic valve. The micromanometer-tipped catheter was manipulated so that the pressure sensor was positioned just distal to the flow probe. Right atrial pressure was measured with a micromanometer-tipped catheter inserted into the cavity through the superior vena cava.

2.2. Chemicals

U-46619 (Cayman Chemical, Ann Arbor, MI, USA) 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. We have previously demonstrated that this vehicle did not influence hemodynamic or aggregometric data [22,26].

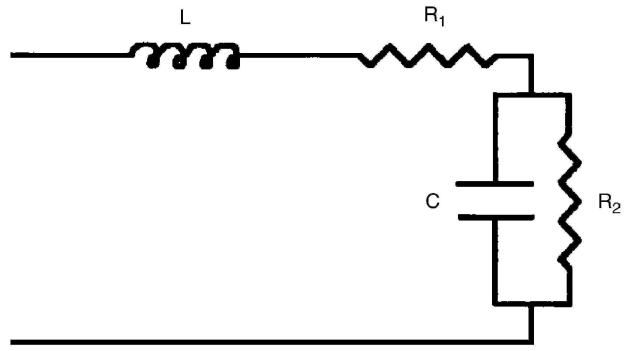
2.3. Experimental protocol

After a 30 min stabilization period, animals were randomly 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 (1.25µg/kg) at 30 min interval. Injections of U-46619 were repeated every 30 min according to specific characteristics of this TP agonist as previously described by Fiedler and collaborators [29]. Briefly, a single intravenous administration of U-46619 (1.25 µg/kg) in pig causes a transient increase in BP. Due to the fast metabolization of this TP agonist, the BP and hemodynamic parameters return to baseline values within 20 min. Consequently, repeated injections of U-46619 every 30 min where chosen because they did not induce cumulative effect overtime. The same protocol was proposed to evaluate dose-dependent effects of thromboxane antagonist Bay-U-3405 in such a model [29]. Hemodynamic data including mean aortic blood pressure (P_{mean}), mean systemic blood flow (Q_{mean}), right atrial pressure, and heart rate (HR), were recorded before each injection of U-46619 (TO), and 2 (T2), and 15 (T15) min after each injection of U-46619.

In the second group (n = 6) (Anta group), an increasing dosage regimen of BM-573 was administrated intravenously 10 min before each U-46619 injection, with the exception of the first one. 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 this group, hemodynamic data were recorded before each antagonist injection (Anta 0, Anta 0.125, Anta 0.250, Anta 0.5, Anta 1, Anta 2), 10 min later (i.e., immediately before agonist injection) (TO), 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 (TO, T2, T15) were called Run 0, Run 0.125, Run 0.250, Run 0.500, Run 1, and Run 2, respectively, according to the dose of antagonist injected in the Anta group.

Fig. 2: *Electric analog of the four-element windkessel model. R_1, characteristic impedance; R_2, peripheral resistance; C, compliance; L, inductance.*



2.4. Data collection

All analog signals were continuously converted to digital form with an appropriate system (Codas, DataQ Instruments Inc., Akron, OH, USA). The pressure and flows 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 an apneic phase and numerically averaged to obtain representative diagrams of pressure and flow waves corresponding to specific experimental conditions.

2.5. Hemodynamic data analysis

Arterial properties were assessed from ascending aortic pressure and flow measurements, and represented with a four-element windkessel model (WK4) [30]. An electrical analog of the WK4 is displayed in Fig. 2. In this model, the resistor R_2 represents the resistive properties of the systemic bed, 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 systemic vessels. The resistor R_1 represents the characteristic impedance, which level depends prominently on the elastic properties and dimensions of the proximal aorta. Finally, an inductance L is introduced to take blood inertia into account. Furthermore, the inductance restores positive phase angles at high frequencies of the impedance spectrum [27].

The values of R_1, R_2, C, and L were estimated by a method previously described [31]. Effective arterial elastance (E_a) was calculated according to the equation:

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

where T_s and T_d are the systolic and diastolic time intervals, respectively. T_s was calculated, in the aortic pressure wave, as the time interval between the point just before the abrupt rise and the dicrotic notch.

2.6. Ex vivo platelet aggregation study and BM-573 measurements

The antiplatelet potency of BM-573 was determined according to a previously described method [32]. The pigs were not treated with aspirin or NSAIDs. 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 a four-channel aggregometer (bioData Corporation, PAP4) [33]. 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) ADP (5 µM final) or (1 µl) collagen (1 µg/ml 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. BM-573 measurements were performed on blood samples by using a high-performance liquid chromatography (HPLC) technique.

2.7. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Between-group comparisons at specific time points, as well as within-group tests for statistical differences at different time points, were performed using a non-parametric and two-sided Wilcoxon (Mann-Whitney) test.

A 5% significance level was used. Reported *P*-values smaller than 0.05 therefore indicate either a between- or a within-group difference (according to the situation under study).

3. Results

3.1. Conventional hemodynamic variables

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

3.1.1. Ago group

From Run 0 to Run 0.250, FIR was not significantly changed after injection of U-46619; in Run 0.5, it showed a particular evolution which was a continuous increase from T0 to T15 ($P = 0.14$); HR increased significantly ($P<0.05$) from 129 ± 10 beats/min (T0) to 145 ± 9 beats/min (T2) in Run 1, and from 119 ± 3 (T0) to 162 ± 15 beats/min (T2) in Run 2.

Q_{mean} decreased from 78 ± 1.8ml/s (T0) to 62.6 ± 2.2ml/s (T2) ($P<0.05$) after administration of U-46619 in Run 0. This decrease worsened over time, and Q_{mean} decreased in the last Run from 61.6 ±1.8 ml/s (T0) to 55.6 ±2.2 ml/s (T2) ($P<0.05$).

P_{mean} did not significantly change after administration of U-46619 during Run 0 to Run 2.

3.1.2. Anta group

There was no difference between Ago and Anta groups during Run 0 for all conventional hemodynamic variables.

HR and Q_{mean} remained unchanged after the injection of U-46619 which followed BM-573 injection in each Run.

P_{mean} did not significantly change after the administration of U-46619 during Run 0 to Run 2.

3.2. Systemic hemodynamic

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

3.2.1. Ago group

In Run 0, R_2 raised from 0.91 ± 0.09 at T0 to 1.32 ± 0.04 mmHg s/ml after administration of U-46619 (T2) (P<0.05), and did not fully return to baseline value at T15 (P<0.05). R_2 increased from T0 to T2 and returned to baseline values at T15 during the following runs.

E_a followed the same pattern as R_2. It increased at T2 after injection of U-46619 and returned to baseline values at T15, except in the last run.

C and R_1 decreased during the first runs after administration of U-46619 (T2) (P<0.05) and did not significantly change in the other runs.

3.2.2. Antα group

From Run 0.250 to Run 2, R_2 and E_a were not influenced by the injection of U-46619.

C and R_1 decreased during the first runs after administration of U-46619 (T2) and did not significantly change in the other runs.

3.3. Ex vivo platelet aggregation study and BM-573 measurements

The effects of BM-573 were studied on platelet aggregation induced by arachidonic acid, collagen or adenosine diphosphate (ADP). Fig. 5 indicates that both basal conditions and administration of U-46619 (basal + U-46619) had no effects on platelet aggregation induced by the three inducers. The platelet aggregation amplitude was maximal and irreversible. Ten minutes after BM-573 injection of 0.125 mg/kg (Run 0.125), a slight decrease in platelet aggregation amplitude was observed when arachidonic acid was used as inducer while both ADP and collagen dependent aggregations remained maximal. After intravenous administration of 0.250 mg/kg of BM-573 and higher doses (Runs 0.500, 1 and 2), the platelet aggregation induced by arachidonic acid was completely inhibited. During the same Runs, platelet aggregation provoked by the other inducers, ADP and collagen slightly decreased but remained maximal and irreversible.

Plasma concentrations of BM-573 were determined after each injection of BM-573. Results are presented in Fig. 6. BM-573 plasmatic levels increased to reach a maximal concentration of 13.3 µg/ml after Run 2. Thirty minutes after Run 2, plasma concentration of BM-573 decreased to 1.6µg/ml.

***Fig. 3**: Time course of conventional hemodynamic variables in the Ago group (close circle) and the Anta group (open square) before (T0), 2min (T2), and 15min (T15) after U-46619 injection in both Ago and Anta groups and, in Anta group, before BM-573 injection (Anta 0.125, Anta 0.250, Anta 0.500, Anta 1, Anta 2, according to the dose of BM-573 injected (mg/kg)). HR, heart rate; Q_{mean}, mean aortic flow.*

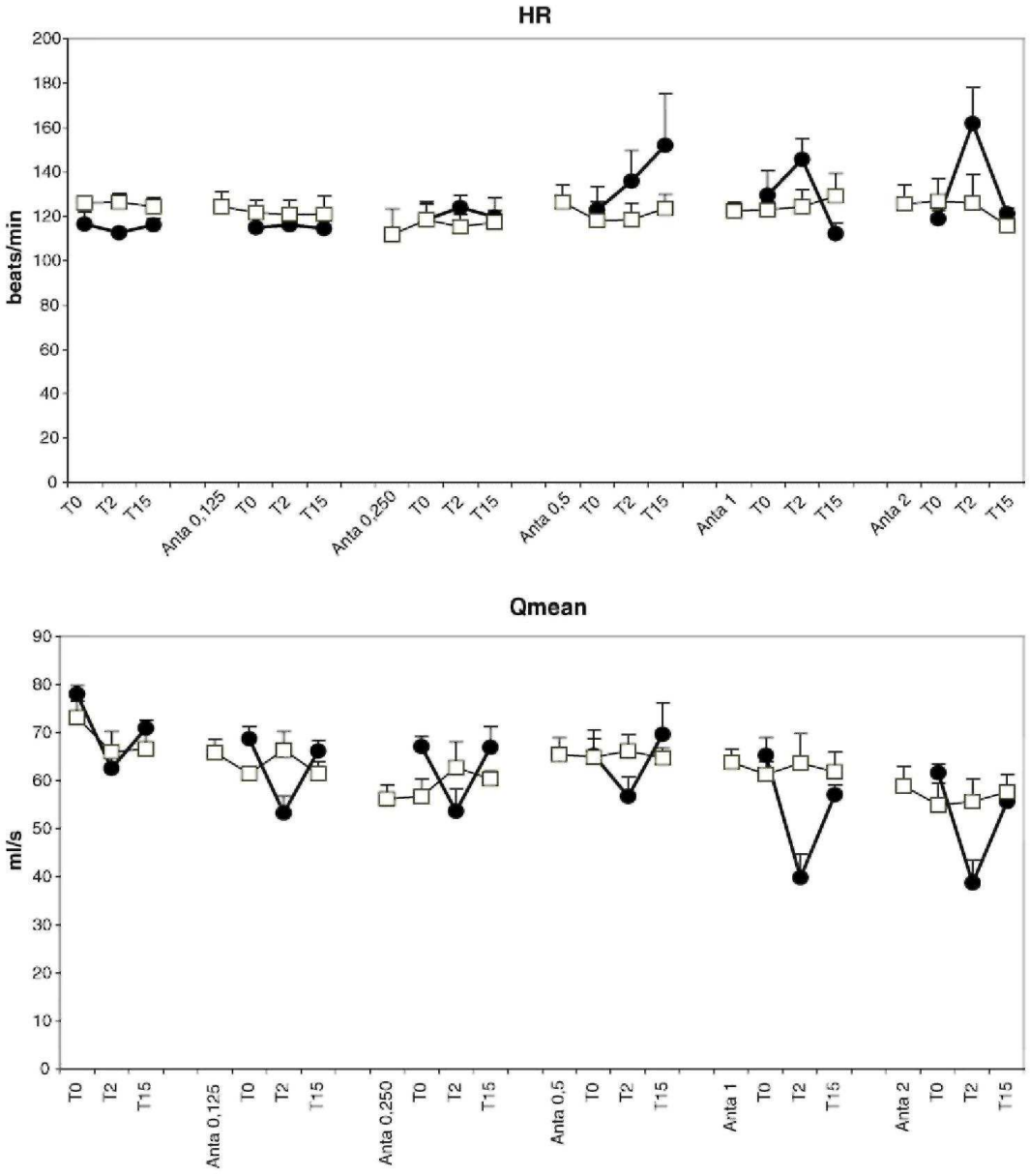


Fig. 4: *Time course of calculated systemic vascular parameters as obtained by the four-element windkessel model of the systemic circulation in the Ago group (close circle) and the Anta group (open square) before (TO), 2 min (T2), and 15 min (T15) after U-46619 injection in both Ago and Anta groups and, in Anta group, before BM-573 injection (Anta 0.125, Anta 0.250, Anta 0.500, Anta 1, Anta 2, according to the dose of BM-573 injected (mg/kg)).* R_2*, peripheral vascular resistance;* E_a*, effective arterial elastance.*

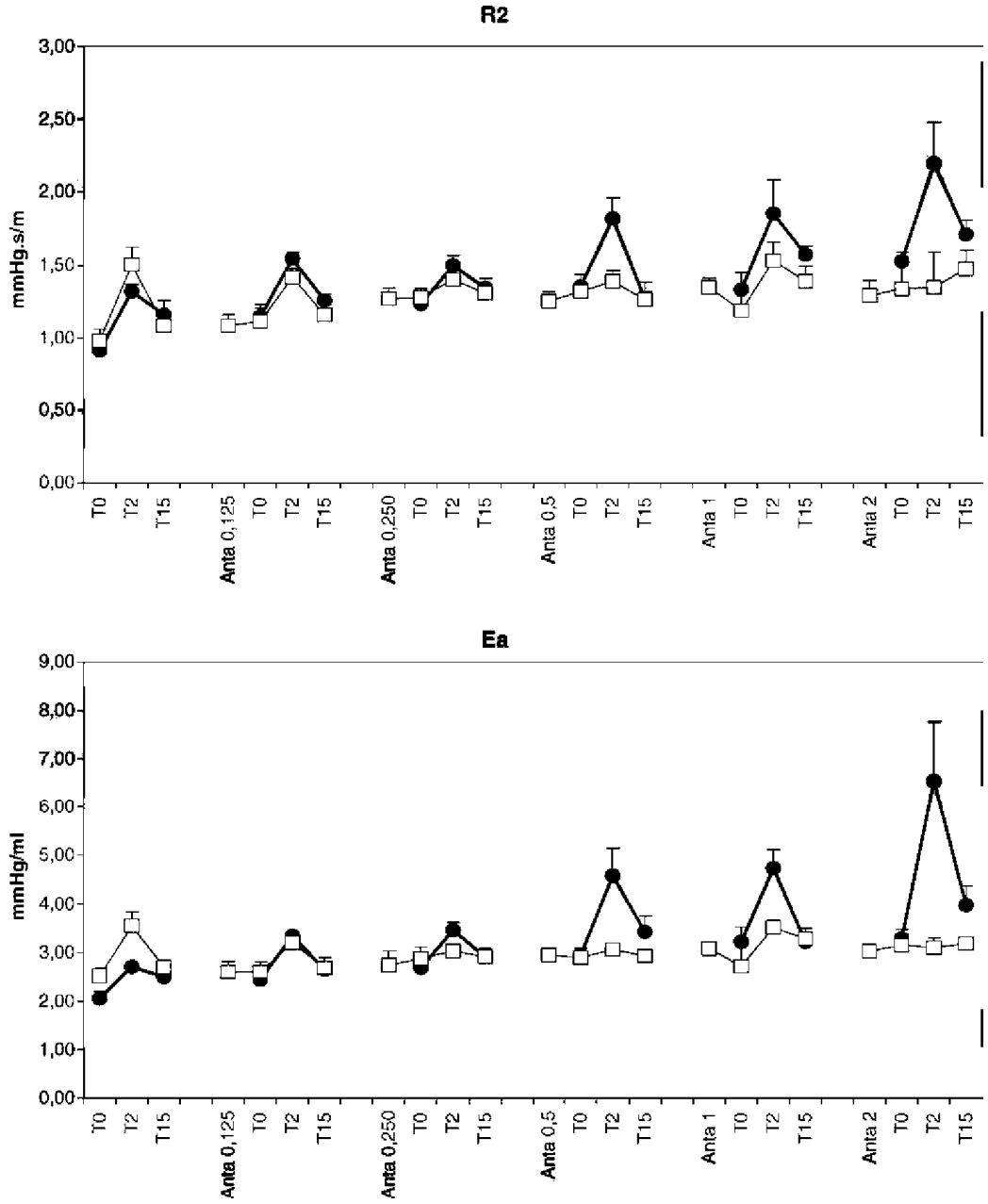


Fig. 5: *Effects of BM-573 on platelet aggregation induced by arachidonic acid, collagen and adenosine diphosphate (ADP).*

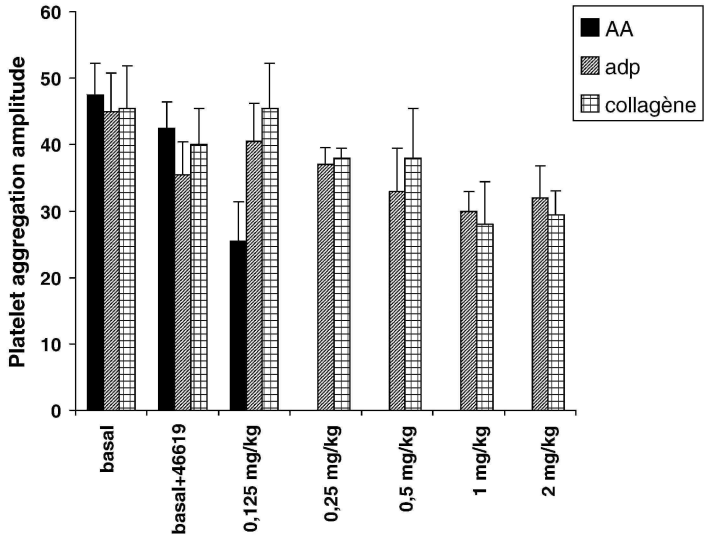
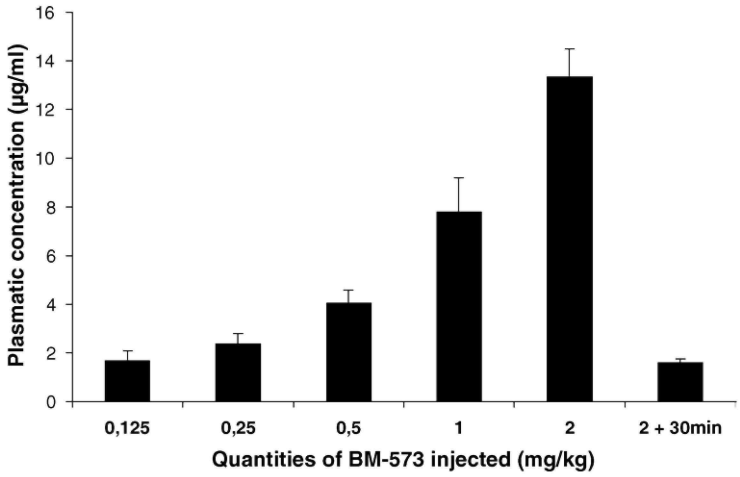


Fig. 6: *Evolution of the plasmatic concentration of BM-573 after each injection.*



4. Discussion

Thromboxane A_2 receptors may be implicated in the regulation of blood pressure and hemodynamic responses in various models of hypertension [13]. Interactions between TXA_2 and the rennin-angiotensin system have also been established, and angiotensin II (Ang II) appeared to stimulate TXA2 synthesis in vascular and renal tissues [16,17]. In the same way, there is evidence for common actions of Ang II and TXA2 to promote systemic and renal vasoconstriction, sodium handling, vascular smooth muscle cell proliferation [18]. These interactions suggest a potential contribution of TXA_2, acting through the TP receptors, to the pathogenesis of hypertension [19]. However, the effects of TXA2 on specific hemodynamic parameters in an in vivo model of systemic hypertension remained undetermined.

In our experiments, we aimed to evaluate the effects of U-46619 on systemic circulatory parameters in the pig before and after administration of BM-573, a thromboxane receptor antagonist and synthase inhibitor. Thus, injection of U-46619 resulted in a significant increase of systemic vascular resistance (R_2), and a decrease in systemic vascular compliance (C). Characteristic impedance (R_1), which reflects the elastic properties of the aorta, was less affected by U-46619 injection despite the decrease noticed in some runs. As a result, arterial elastance (E_a) markedly raised. BM-573 completely blocked the increase of arterial elastance (E_a), and stabilized P_{mean} and Q_{mean} at the regimen of 0.500 mg/kg.

In a previous study using mice lacking TP receptors, intravenous infusion of U-46619 caused transient increase inblood pressure followed by cardiovascular collapse in wild-type mice. However, U-46619 did not modify hemodynamic parameters in TP knock-out mice suggesting that these actions were mediated exclusively via TP receptors [34]. Another study reported that left atrial injections of U-46619 decreased heart rate and arterial blood pressure in the anesthetized rabbit by stimulation of cardiac vagal afferent nerves [35]. In our study, P_{mean} increased during the first runs after U-46619 injection, and then decreased in Runs 1 and 2, a decrease which induced an augmentation of HR. Moreover, administration of U-46619 induced a transient decrease of mean systemic blood flow (Q_{mean}) during all Runs which can be explained by the marked increase in left ventricular afterload (E_a). In a previous study on the effects of proximal aortic banding on left ventricular performance, the same drop of Q_{mean} was noticed when the left ventricle was facing an increase in proximal and distal vascular resistance (R_1 and R_2) [36].

In another study, U-46619 was also responsible for an increase in pulmonary vascular resistance which induced an increase in pulmonary artery pressure [24]. Such an increase in right ventricular afterload can also contribute to the decrease in Q_{mean} by reducing LV preload. In our study, the administration of U-46619 induced an increase of LV afterload which can be mainly explained by its effects on peripheral resistances (R_2), but surprisingly no significant changes occurred in P_{mean} as we should have been expected. This rapid increase of LV afterload resulted in an impairment of LV performance reflected by the drop of Q_{mean} as described in a previous report [36].

Our study also clearly demonstrated that the systemic vascular responses to the TXA_2 stable agonist, U-46619, were significantly reduced by BM-573. Moreover, the intravenous injections of 0.500 mg/ml of BM-573 and higher doses resulted in a complete inhibition of platelet aggregation induced by arachidonic acid. In the same conditions, the platelet aggregation induced by the ADP and collagen was not affected. These results indicate that BM-573 is effective on platelets as specific thromboxane modulator. The platelet amplitude observed within this species appeared to be lower than the platelet amplitude observed in human. Indeed, we also previously evaluated the effects of BM-573 as antiaggregating agent in human PRP (3×10^8 cells ml^{-1}) [21,23]. However, the data remain interpretable and comprehensive and are in accordance with the antiplatelet effects of BM-573 observed in vitro and in vivo in other species [22,23]. In the present study, we also confirmed that BM-573 is rapidly metabolized when injected intravenously since the plasmatic levels were not cumulative. Indeed, we observed that 30 min after the last injection (Run 2) of 2mg/kg of BM-573, the plasma level of this drug decreased significantly (88%). We also demonstrated that BM-573 was a strong ligand of TP receptors and acted as a competitive and selective antagonist of these receptors [21,23]. Consequently, The effects of BM-573 can be explained by its antagonism of U-46619 at the receptor level in a competitive manner. Other details on the pharmacodynamic and the pharmacokinetic parameters of BM-573 have been described in other pharmacological models performed in other animals [22,25,26].

In conclusion, injection of the stable TXA_2 agonist U-46619 induced a rapid and transient augmentation in LV afterload, responsible for LV impairment, as evidenced by a drop in cardiac output, which is prevented by BM-573. Furthermore, these data confirmed that BM-573 is able to block TXA_2 receptors present in the systemic circulation. BM-573 could therefore be a promising therapeutic approach in pathophysiological states where TXA_2 appears to play a main role in the increase of vascular resistance as it has recently been suggested in hypertension.

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