THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 309, No. 2

63610/1141110
JPET 309:498–505, 2004
*Printed in U.S.A.*

# Pharmacological Characterization of *N-tert*-Butyl-*N′*-[2-(4′-methylphenylamino)-5-nitrobenzenesulfonyl]urea (BM-573), a Novel Thromboxane $A_2$ Receptor Antagonist and Thromboxane Synthase Inhibitor in a Rat Model of Arterial Thrombosis and Its Effects on Bleeding Time

Jean-Michel Dogné, Julien Hanson, Xavier de Leval, Philippe Kolh, Vincent Tchana-Sato, Laurence de Leval, Stéphanie Rolin, Alexandre Ghuysen, Patrick Segers, Bernard Lambermont, Bernard Masereel, and Bernard Pirotte

*Natural and Synthetic Drug Research Center, University of Liège, Liège, Belgium (J.-M.D., J.H., X.d.L., B.P.); Experimental Hemodynamics Laboratory (HemoLiège) (P.K., V.T.-S., A.G., B.L.) and Department of Pathology (L.d.L.), University Hospital of Liège, Liège, Belgium; Department of Pharmacy, University of Namur, Namur, Belgium (S.R., B.M.); Hydraulics Laboratory, Institute of Biomedical Technology, Ghent University, Ghent, Belgium (P.S.)*

Received November 28, 2003; accepted January 16, 2004

## ABSTRACT

The present study was undertaken to characterize the antiplatelet and antithrombotic effects of BM-573 [*N-tert*-butyl-*N′*-[2-(4′-methylphenylamino)-5-nitrobenzenesulfonyl]urea], an original combined thromboxane receptor antagonist and thromboxane synthase inhibitor in rats, and to determine its effects on mice bleeding time. Intraperitoneal injection of a single dose of 5 mg/kg BM-573 to rats inhibited U-46619 (9,11-dideoxy-9,11-methanoepoxy-prostaglandin $F_2$)-induced washed platelet aggregation 30 min and 1, 2, and 4 h after drug administration with a maximum antiplatelet effect observed after 1 and 2 h. In a rat model of thrombosis induced by ferric chloride application on the abdominal aorta, BM-573 significantly reduced the thrombus weight by 92.53, 80.20, 64.75, and 18.21% at doses of 5, 2, 0.5, and 0.2 mg/kg, respectively. Time to occlusion of abdominal aorta in the BM-573-treated group (41.50 ± 5.21 min) was significantly prolonged compared with the vehicle-treated rats (16.16 ± 0.79 min). Like furegrelate, seratrodast, and acetylsalicylic acid, BM-573 did not affect the tail bleeding time induced by tail transection in mice compared with vehicle-treated mice. Moreover, BM-573, a close derivative of the loop diuretic torasemide, failed to induce a significant increase in diuresis in rat and did not produce a decrease in blood glucose concentration as observed with the sulfonylurea glibenclamide. In conclusion, we have demonstrated that the nitrobenzenic sulfonylurea BM-573, an original combined thromboxane receptor antagonist and thromboxane synthase inhibitor, is a potent antithrombotic agent that does not affect bleeding time. Moreover, BM-573 lost the diuretic property of torasemide and has no impact on glycemia.

The isozymes cyclooxygenase (COX)-1 and -2 catalyze the conversion of arachidonic acid into thromboxane $A_2$ ($TXA_2$) and prostaglandins (PGs). The eicosanoid $TXA_2$ is the major COX-1 product of arachidonic acid metabolism in platelets. $TXA_2$ is formed by the action of thromboxane synthase on the prostaglandin endoperoxide $H_2$ ($PGH_2$) and causes vasoconstriction, bronchoconstriction, and irreversible platelet aggregation (Hamberg et al., 1975; Svensson et al., 1976; Bhagwat et al., 1985; Fiddler and Lumley, 1990). Furthermore, recent studies have pointed out a significant stimulatory role of $TXA_2$ in the proliferation of vascular smooth muscle cells and mitogenesis (Pakala and Benedict, 1998; Koba et al., 2000). Its biosynthesis is increased in syndromes of platelet activation, such as myocardial infarction (Eikelboom et al.,

This work was supported by Fonds de la Recherche Scientifique Médicale Grant 3.4.505.01.F. Vincent Tchana-Sato is a Research Fellow of the Fonds National de la Recherche Scientifique (FNRS), and Laurence de Leval is a Research Associate of the FNRS.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.103.063610.

**ABBREVIATIONS:** COX, cyclooxygenase; $TXA_2$, thromboxane $A_2$; ASA, aspirin; PG, prostaglandin; $PGH_2$, prostaglandin endoperoxide $H_2$; TP, $TXA_2$ receptor; $PGI_2$, prostacyclin; BM-573, *N-tert*-butyl-*N′*-[2-(4′-methylphenylamino)-5-nitrobenzenesulfonyl]urea; U-46619, 9,11-dideoxy-9,11-methanoepoxy-prostaglandin $F_2$; BT, bleeding time; HPLC, high-performance liquid chromatography; TTO, time to occlusion; TXRA, thromboxane receptor antagonist; TXSI, thromboxane synthase inhibitor; Z-335, [2-(4-chlorophenylsulfonylaminomethyl)indan-5-yl]acetate; BMS-180291, [(+)1*S*-(1$\alpha$,2$\alpha$,3$\alpha$,4$\alpha$)]-2-[[3-[4-[(n-pentylamino)carbonyl]-2-oxazolyl]-7-oxabicyclo[2.2.1]hept-2-yl]methyl]benzenepropanoic acid].

2002), unstable angina (Hamm et al., 1987), and thrombosis and thrombotic disorders (Saldeen et al., 1993), but also in other pathophysiological states, such as pulmonary hypertension, asthma, and septic shock (Dogné et al., 2003). Inhibition of thromboxane synthesis underlies the efficacy of aspirin (ASA) in significantly reducing the incidence of cardiovascular death, myocardial infarction, and stroke in high-risk patients (Collins et al., 1994). Initially, there was great interest in the potential use of $TXA_2$ receptor (TP) antagonists such as sulotroban; however, these compounds were too selective to completely inhibit platelet aggregation. In general, interest has switched to agents that are combined receptor antagonists and thromboxane synthase inhibitors, such as ridogrel and terbogrel. The rationale behind this switch is that the TP receptor antagonist activity can block the aggregatory and vasoconstrictor actions of both $TXA_2$ and $PGH_2$. Moreover, the endoperoxide that accumulates after inhibition of thromboxane synthesis can be converted to either $PGD_2$ by the platelets or prostacyclin ($PGI_2$) by the vessel wall, both of which increase platelet cyclic AMP levels and inhibit platelet activation (Cheng et al., 2002, de Leval et al., 2003, Dogné et al., 2004) (Fig. 1).

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 nitrobenzene and the presence of a *tert*-butyl group on the distal nitrogen atom of the sulfonylurea moiety (Fig. 2). These modifications improved $TXA_2$ antagonism and revealed $TXA_2$ synthase inhibitory potency. Indeed, we demonstrated that this original molecule showed a high affinity ($IC_{50}$, 1.3 nM) for the TP receptors of human platelets. Moreover, BM-573 was found to be a potent inhibitor of human platelet aggregation induced by arachidonic acid ($ED_{100}$, 0.13 $\mu$M) or by the stable $TXA_2$ mimetic U-46619 ($ED_{50}$, 0.24 $\mu$M). BM-573 also relaxed the isolated rat thoracic aorta ($ED_{50}$, 28.4 nM) contracted by U-46619 and completely reduced the platelet production of the thromboxane $B_2$, the stable $TXA_2$ metabolite in blood, induced by arachidonic acid (Rolin et al., 2001).

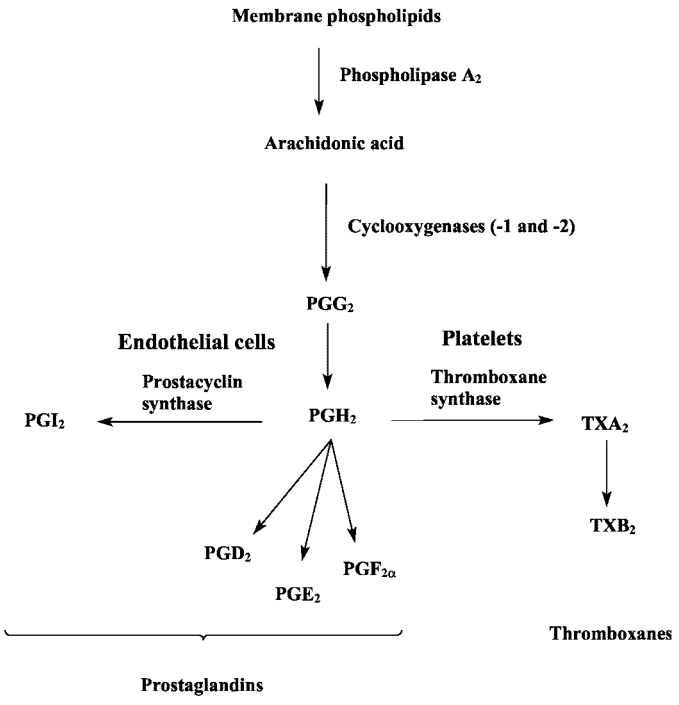



**Fig. 1.** Arachidonic acid cascade and the cyclooxygenase pathway. $TXA_2$ is formed by the action of thromboxane synthase on the $PGH_2$ mainly in activated platelets where this enzyme is highly expressed. In platelets, $PGH_2$ is the result of the enzymatic action of the constitutive form of COX-1 on arachidonic acid released from the cell membrane phospholipids by phospholipase $A_2$. In endothelial cells, prostacyclin synthase can convert $PGH_2$ into $PGI_2$.

These promising in vitro pharmacological properties led us to study the pharmacological profile of BM-573 in vivo as an antiplatelet and antithrombotic agent. Thus, we measured the blood concentration of BM-573 following intraperitoneal administration in rats at different times and evaluated the effects on ex vivo platelet aggregation. We determined the antithrombotic activity of our drug in a ferric chloride abdominal arterial thrombosis model in rat and evaluated its effects on bleeding time (BT) in mice. Finally, we studied the effects of the intraperitoneal injection of BM-573 in rat on diuresis and glycemia and compared them with those induced by torasemide and the sulfonylurea glibenclamide, respectively.

## Materials and Methods

**Drugs.** BM-573 and torasemide [*N*-propyl-*N*′-[(4-m-toluidino-3-pyridyl)sulfonyl]urea] were synthesized in our laboratory. The sodium salts were dissolved in propyleneglycol and diluted with physiological saline. Furegrelate [5-(3-pyridinylmethyl)-2-benzofurancarboxylate] and U-46619 were purchased from Cayman Chemical (Ann Arbor, MI). They were dissolved in ethanol and diluted with physiological saline. Acetylsalicylic acid and glibenclamide were purchased from Sigma-Aldrich (Bornem, Belgium). Seratrodast was isolated from Bronica, a generous gift from Takeda Chemical Industries (Osaka, Japan). The sodium salts were dissolved in propyleneglycol and diluted with physiological saline.

**Animals.** Male Sprague-Dawley rats weighing 250 to 300 g and 8- to 12-week-old male and female mice were housed in a temperature-controlled room before being used in the present experiments. All experimental procedures and protocols used in this investigation have been carried out in accordance with the Declaration of Helsinki (Publication 85-23, revised 1985) and were reviewed and approved by the Ethics Committee of the Medical Faculty of the University of Liège (Liège, Belgium).

**Ex Vivo Platelet Aggregation in Rat and BM-573 Measurements.** Male Sprague-Dawley rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood (2.5 ml) was drawn from the abdominal aorta into a tube containing 0.3 ml of trisodium citrate (3.2% w/v) as an anticoagulant. Platelet-rich plasma was prepared by centrifugation at 180*g* for 10 min (15°C). Platelet-poor plasma was obtained by centrifugation of remaining blood at 2205*g* for 10 min (15°C). The platelet-aggregation method was modified from Yokoyama et al. (1994). Briefly, platelet-rich plasma was centrifuged at 1000*g* for 20 min, and the supernatant was discarded. The platelets were suspended in washing buffer at a volume equal to the original plasma volume and centrifuged at 1000*g* for 20 min. The platelets were resuspended in a suspension buffer and adjusted to a concentration of $5 \times 10^8$ cells/ml.

The washing buffer contained 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM $NaH_2PO_4$, 0.05 trisodium citrate, and 0.1% glucose (w/v). The pH was adjusted to 6.5 with HCl. The suspension buffer contained 113 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, 24 mM $NaHCO_3$, 10 mM HEPES-NaOH (pH 7.4), and 0.1% glucose (w/v) (Li et al., 1998).

Platelet aggregation was studied using the turbidimetric method of Born (Born and Cross, 1963) in an aggregometer Chronolog Corporation (Chicago, IL) as previously described (Loï et al., 1998). A volume of 240 $\mu$l of the platelet suspension was placed in a glass

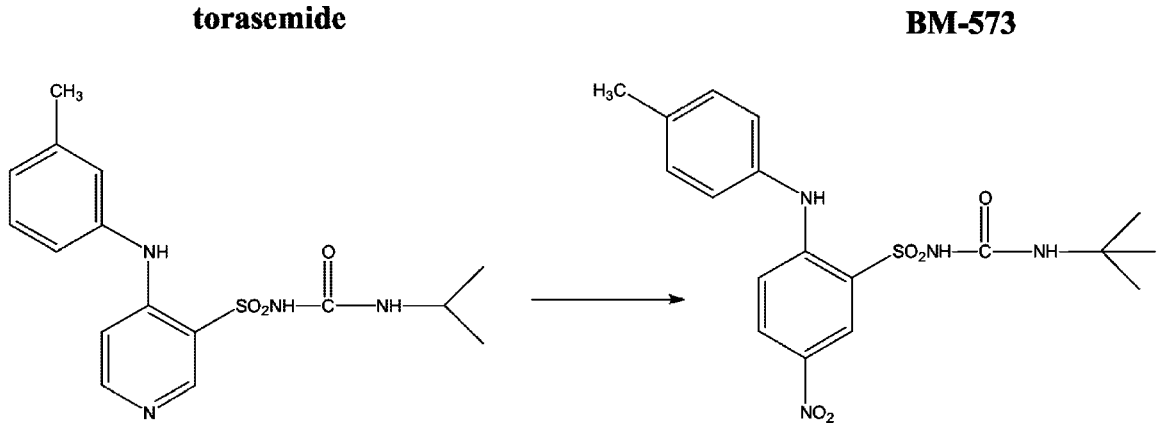



**Fig. 2.** Chemical structures of the pyridinic sulfonylurea torasemide and the nitrobenzenic sulfonylurea BM-573.

turbidity tube and warmed at 37°C for 3 min in the cells of the aggregometer. Three minutes later, 2 $\mu$l of calcium chloride solution was added to obtain a final concentration of calcium equal to 0.2 mM. One minute later, 10 $\mu$l of U-46619 (final concentration of 1 $\mu$M) was added. Changes in light transmission were recorded 10 min after stimulation with U-46619. The extent of aggregation was estimated by the percentage of maximum increase in light transmission, with the buffer representing 100% transmittance.

To examine the duration of the inhibitory effects of the BM-573 on platelet aggregation, the drug was administered intraperitoneally in rats 30 min and 1, 2, 4, 7, and 10 h prior to blood (2.5 ml) being drawn from the abdominal aorta. BM-573 measurements were performed on blood samples at the same times by using a high-performance liquid chromatography (HPLC) technique.

### Ferric Chloride-Induced Rat Arterial Thrombosis

**Thrombus Weight and Histopathology.** The experiments were carried out according to the modification of the method described by Kurz et al. (1990). Rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). After an abdominal midline incision, the abdominal aorta was carefully exposed. A filter paper disk (diameter 8 mm) saturated with 50% (w/v) ferric chloride solution was placed on the surface of the artery for 10 min. The artery was isolated 10 min after removing the disk, and then the rat was euthanized. The removed abdominal artery was opened lengthwise, and the thrombus was scraped out and placed on filter paper to remove any water. Its wet weight was measured immediately. Results are expressed in milligrams of thrombus weight by kilograms of rat weight. BM-573 (5, 2, 0.5, 0.2 mg/kg), placebo, torasemide (5 mg/kg), and furegrelate (5 mg/kg) were injected intraperitoneally 1 and 2 h prior to the application of ferric chloride.

Cross-sections of abdominal rat aorta treated with the paper filter soaked with a solution of ferric chloride as described above were fixed overnight in buffered formalin and embedded in paraffin. Four-micron sections were cut and stained with hematoxylin and eosin.

**Rat Abdominal Aortic Blood Flow Measurements.** A filter paper disk (diameter 8 mm) saturated with 50% (w/v) ferric chloride solution was placed on the surface of the rat artery for 10 min following the same procedure as described above. The rat abdominal aortic blood flow expressed in milliliters/minute was recorded continuously by an ultrasonic Doppler flow probe (Transonic Systems Inc., Ithaca, NY). The time to occlusion (TTO) of the abdominal aorta was measured in minutes.

**Bleeding Time in Mice.** Bleeding times were assessed according to Ma et al. (2001). Briefly, mice were placed in a holder, and their tails were transacted with a surgical blade 1 cm proximal from the tip. The remaining tail was immersed immediately into physiological saline maintained at 37°C, and the time during which visible bleeding was observed was measured. BM-573, furegrelate, seratrodast, aspirin, ticlopidine, and vehicle were given intraperitoneally 1 h prior to tail cutting.

**Diuresis.** Experiments were conducted as described previously (Masereel et al., 1993). Rats (250–300 g) received an intraperitoneal injection of BM-573 or torasemide at the dose of 5 mg/kg. Rats were allowed free access to food and water until the beginning of the experiment and were housed in groups of three in metabolism cages. Urine was collected for 4 h after drug administration, and diuresis (milliliters per kilogram) was expressed as the mean of urinary volume (milliliters) collected from the cage.

**Glycemia.** The methodology was performed according to Lebrun et al. (2000). Briefly, rats were allowed to settle in the laboratory for at least 3 days before use. They had free access to water and received a standard pellet diet. Conscious rats were placed in a small cabinet 60 min prior to blood sampling and throughout the duration of the study. At zero time, BM-573 or glibenclamide were administered intraperitoneally at a dosage of 20 mg/kg. Control animals received an equivalent volume of water and propyleneglycol (50% total volume; 1.5 ml/kg body weight). Blood was taken from the tail at time 5, 30, 60, 120, and 180 min. Blood glucose was measured in duplicate using a reagent strip in combination with a glucometer (Gluco Touch; LifeScan, a Johnson & Johnson Company, Beerse, Belgium).

**Statistical Analysis.** Results are expressed as the mean $\pm$ S.E.M., and statistical significance was determined by Student's $t$ test and the chi-square test. Probability values of less than 0.05 were considered to be significant.

## Results

**Ex Vivo Platelet Aggregation in Rat and BM-573 Blood Measurements.** Intraperitoneal injection of a single dose of 5 mg/kg BM-573 to rats inhibited U-46619-induced washed platelet aggregation 30 min and 1, 2, and 4 h after drug administration. The maximum antiplatelet effect was observed 1 and 2 h after BM-573 administration (Fig. 3). Blood levels of BM-573 measured by HPLC were maximum 1 h after BM-573 injection and decreased time-dependently after this period (Fig. 4).

### Antithrombotic Effect of BM-573

**Thrombus Weight.** The application of ferric chloride (50% w/v) for 10 min to the abdominal aorta induced marked thrombi in vehicle-treated rats (8.3973 $\pm$ 0.369 mg/kg). In this group, the mixed thrombi composed of both fibrin and platelets were adherent to the vessel wall at the site of ferric chloride contact, as revealed by light micrographs of abdominal rat aorta sections stained with hematoxylin-eosin (Fig. 5).

The intraperitoneal injection of BM-573 at doses of 5, 2,

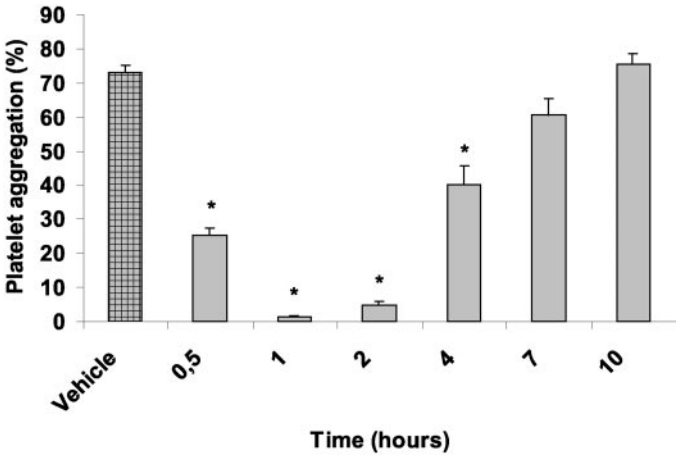



**Fig. 3.** Time-dependent effect of BM-573 on platelet aggregation induced in rat washed platelets by U-46619. Each column represents the mean ± S.E.M. of 6 to 7 rats. *, $P < 0.05$ compared with the vehicle-treated group.

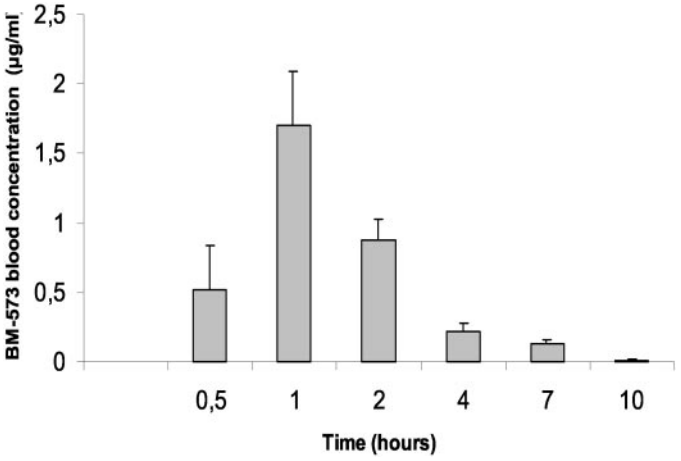



**Fig. 4.** Blood levels of BM-573 ($\mu$g/ml) determined by HPLC. Mean ± S.E.M. ($n = 6$). *, $P < 0.05$ compared with the vehicle-treated group.

0.5, and 0.2 mg/kg 1 h before ferric chloride application resulted in a significant reduction of thrombus weight by 92.53, 80.20, 64.75, and 18.21%, respectively. When BM-573 was administered intraperitoneally 2 h before ferric chloride treatment at doses of 5 and 2 mg/kg, the thrombus weight was reduced by 61.34 and 44.58%, respectively. No significant effects were observed with torasemide, furegrelate, or seratrodast on thrombus formation when administered intraperitoneally at 5 mg/kg 1 or 2 h before thrombus induction (Fig. 6).

**Time to Occlusion.** TTO of the abdominal aorta in vehicle-treated rats ranged from 13 to 18 min after the filter paper was soaked in a 50% solution of $FeCl_3$ application with an average TTO of 16.16 ± 0.79 min. After an intraperitoneal injection of 5 mg/kg BM-573 1 h before ferric chloride application, the average TTO measured was 41.50 ± 5.21 min. In the same conditions, torasemide, seratrodast, and furegrelate did not prolong TTO.

**Effect of BM-573 on Bleeding Time in Mice.** BM-573, furegrelate, seratrodast, and acetylsalicylic acid (5 mg/kg, i.p., 1 h pretreatment) did not affect the tail BT induced by tail transection in mice compared with vehicle-treated mice. Aspirin tended to prolong the tail bleeding time, although its effect failed to reach significance. In the same conditions, ticlopidine significantly prolonged BT up to 247 s (Fig. 7).

**Diuretic Properties of BM-573 in Rat.** Diuretic properties of BM-573 and torasemide were studied time-dependently after intraperitoneal administration of 5 mg/kg in rat. Figure 8 shows that the single intraperitoneal administration of torasemide induced a significant increase in diuresis in rat. Cumulated urine volumes measured in the BM-573-treated group do not differ from those in the vehicle-treated group.

**Effects of BM-573 on Blood Glucose Concentration.** In control rats receiving the vehicle, the blood glucose level was stable for 180 min. The glycemia averaged 115.2 ± 9.7 mg/100 ml at the 5th min and 109.4 ± 16.6 mg/100 ml at the 180th min. The intraperitoneal injection of BM-573 did not provoke a decrease in glycemia. The blood glucose level amounted to 116.6 ± 12.3 mg/100 ml at the 5th min and 105.2 ± 11.9 mg/100 ml at the 180th min. The hypoglycemic effect of glibenclamide observed was rapid and marked. At the 60th min, the glycemia averaged 67.4 ± 7.8 mg/100 ml after the administration of 20 mg/kg (Fig. 9).

## Discussion

Thromboxane $A_2$ is both a powerful aggregating mediator and a constrictor of blood vessels. $TXA_2$ levels are elevated in conditions associated with platelet activation leading to thrombosis, including unstable angina, myocardial infarction, and cerebral ischemia. Aspirin is the most common drug used to prevent $TXA_2$ production. Indeed, low doses of aspirin selectively inhibit the formation of $TXA_2$ via cyclooxygenase-1 inhibition without altering the basal biosynthesis of cardioprotective prostacyclin produced via cyclooxygenase-2 by endothelial cells. Furthermore, ASA causes complete enzyme inhibition, without the recovery of enzyme activity. The effect of ASA in the prevention of ischemic events has been well documented in many older and recent clinical trials. It is clear from these studies that ASA, alone or in combination with other antithrombotics, significantly reduces the incidence of cardiovascular death, stroke, and myocardial infarction (Mangano, 2002; Gorelick PB et al., 2003). Thus, acetylsalicylic acid has been a drug of choice as an antiplatelet agent; however, aspirin sensitivity has been noted as leading to asthma and Reye's syndrome (Pinsky et al., 1988). Moreover, ASA use is associated with gastroduodenal mucosal damage and increased risk of upper gastrointestinal bleeding (Awtry and Loscalzo, 2000). Since inhibition of thromboxane synthesis underlies the efficacy of aspirin, specific thromboxane receptor antagonists (TXRAs) such as seratrodast and thromboxane synthase inhibitors (TXSIs) such as ozagrel have been developed. Both thromboxane receptor antagonists and thromboxane synthase inhibitors show interesting pharmacological characteristics. TXSIs can result in an increase of the synthesis of the antiaggregatory and vasodilatory prostacyclin, and TXRAs can block the action of both $TXA_2$ and $PGH_2$ at a receptor level. A solution to thereby optimize the therapeutic benefit of both types of agents used alone was to combine their properties in the same molecule. Indeed, to support this concept, a series of double-blind, placebo-controlled, crossover studies in healthy volunteers was carried out using a coadministration of the TXRA sulotroban and the TXSI dazoxiben. Results obtained con-

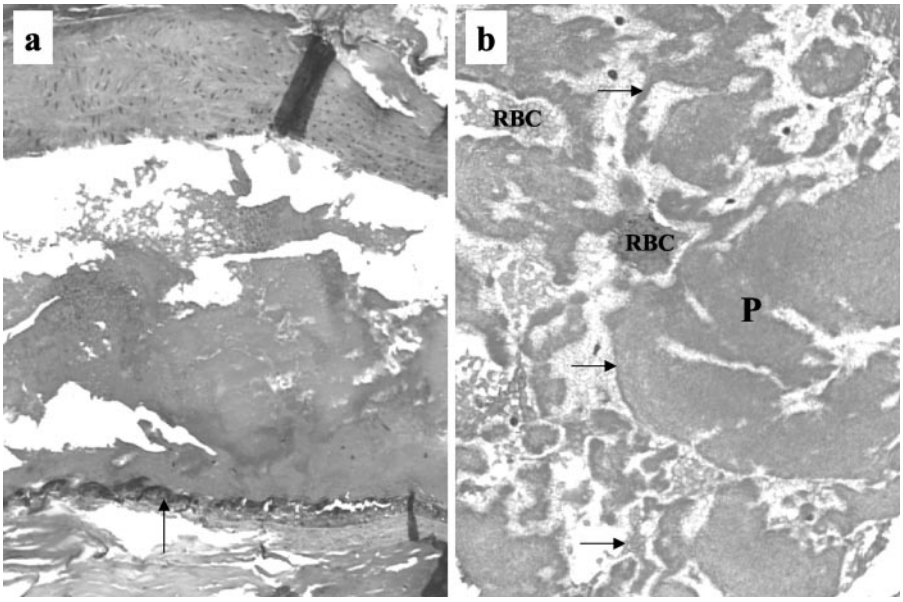
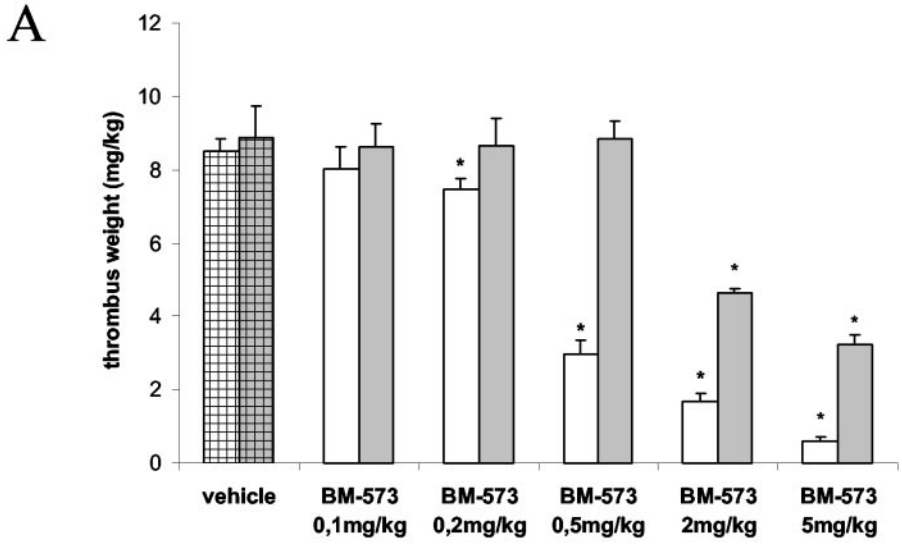



**Fig. 5.** A, longitudinal section of an aorta showing a subocclusive thrombus induced by $FeCl_3$ (brown linear deposit, arrow) (hematoxylin and eosin, original magnification ×50). B, high-power view of the thrombus showing a meshwork of fibrin (arrows) associated with large aggregates of platelets (P) and red blood cells (RBC) (hematoxylin and eosin, original magnification ×200).



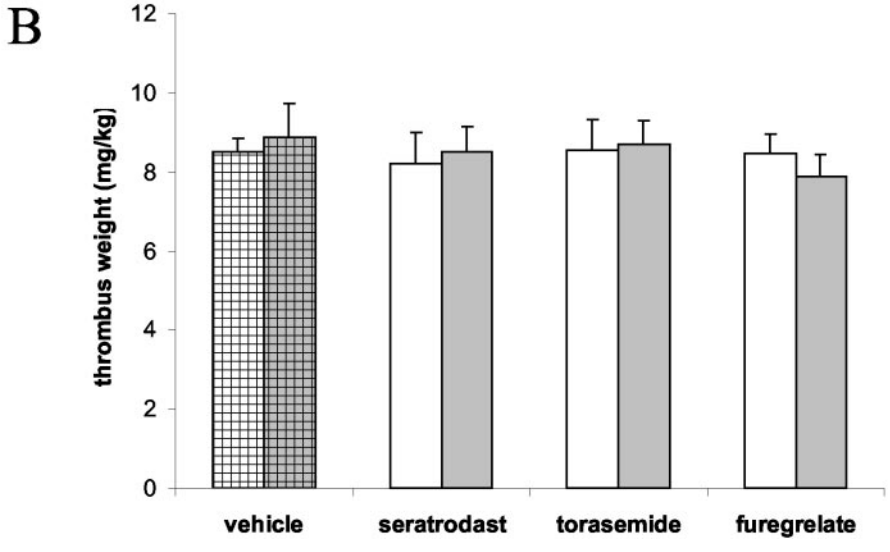




**Fig. 6.** A, dose-dependent effects of BM-573 on thrombus weight (milligrams per kilogram) 1 (white columns) and 2 h (gray columns) before ferric chloride application on rat abdominal aorta. B, effects of seratrodast, torasemide, and furegrelate (5 mg/kg, i.p.) on thrombus weight (milligrams per kilogram) 1 (white columns) and 2 h (gray columns) before ferric chloride application on rat abdominal aorta. Each column represents the mean ± S.E.M. of 6 to 7 rats. ∗, $P < 0.05$ compared with the vehicle-treated group.

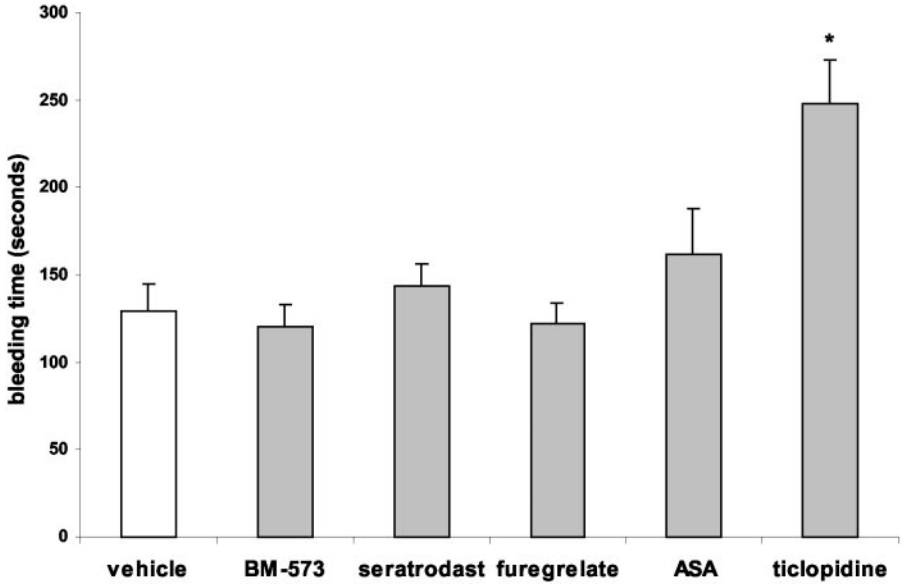



**Fig. 7.** Effect of BM-573, furegrelate, seratrodast, acetylsalicylic acid, and ticlopidine (5 mg/kg, i.p., 1 h pretreatment) on tail bleeding time in mice. Mean ± S.E.M. ($n = 6$). *, $P < 0.05$ compared with the vehicle-treated group.

firmed the greater interest for coadministration of both thromboxane modulators than their independent use (Dogné et al., 2000, 2003).

In previous in vitro and ex vivo studies, it has been demonstrated that BM-573 was a potent combined compound capable of reducing $TXA_2$ production by thromboxane inhibition and preventing the action of $TXA_2$ (or $PGH_2$) by blocking the $TXA_2$ receptors. The present study demonstrated that this dual $TXA_2$ receptor antagonist and thromboxane synthase inhibitor prevents platelet aggregation and thrombus formation without affecting bleeding time. Indeed, intraperitoneal injection of BM-573 (5 mg/kg) to rats inhibited U-46619-induced washed platelet aggregation. The maximum antiplatelet effect was observed 1 and 2 h following BM-573, corresponding to the higher blood levels of BM-573 assayed by HPLC. This result indicates that BM-573 is active as TXRA in vivo and is rapidly metabolized after i.p. injection. Indeed, BM-573 blood levels decrease significantly 2 h following the injection, and the ex vivo antiplatelet effect cannot be observed after 7 h. In support of these pharmacokinetic data, we evaluated the antithrombotic effect of BM-573 on ferric chloride-induced arterial thrombosis in rats. We chose this model because the development of thrombi in response to ferric chloride-induced vascular injury was described to be physiologically relevant (Kurz et al., 1990). In this model, we observed that the application of ferric chloride to the abdominal aorta induced marked mixed thrombi composed of activated platelets, fibrin strands, and entrapped erythrocytes. These thrombi were adherent to the vessel wall at the site of ferric chloride contact, as revealed by light micrographs of abdominal rat aorta sections (Fig. 5). By using a ferric-chloride solution concentration of 50% w/v, the time to form an occlusive thrombus that induced the occlusion of rat abdominal aorta was less than 20 min in all experiments, with an average of 16.16 min. This result is in accordance with the data of Tanaka and collaborators, who performed a similar thrombus model in rats (Tanaka et al., 2000). In our study, BM-573 clearly prevented thrombus formation following ferric chloride-induced vascular injury, with maximum activity observed when injected 1 h before thrombus formation. Both the histopathological examination and time to occlusion measurements confirmed this antithrombotic effect. Moreover, the TXRA seratrodast and TXSI furegrelate were inactive. This result confirms our previous data supporting that seratrodast, an antiasthmatic agent, was a weak human platelet thromboxane receptor antagonist (Dogné et al., 2003). Furegrelate is a weak TXSI that only prevents human platelet aggregation induced by arachidonic acid or U-46619 at high concentrations. These findings suggest that potent thromboxane modulators such as BM-573 are effective in preventing acute and mixed-type arterial thrombosis. Our results are supported by two previous reports indicating that, in a similar rat model of ferrous chloride-induced artery thrombosis, two selective TP antagonists, Z-335, given orally, and BMS-180291, given intravenously, decreased the weight of arterial thrombi (Tanaka et al., 2000). We thereafter examined the effects of BM-573 at the antithrombotic dose of 5 mg/kg on bleeding time in mice compared with seratrodast, furegrelate, aspirin, and ticlopidine. The bleeding time measured in the vehicle-group (129.3 ± 15.4 s) was coherent with results obtained by Ma et al. (2001). In this test, all thromboxane modulators failed to prolong bleeding time. Aspirin tended to prolong the tail bleeding time, although its effect was not statistically significant. These results confirm that $TXA_2/PGI_2$ balance is not a major factor in the regulation of bleeding time in acute models (Tanaka et al., 1998). The causes of the separation of antithrombotic and bleeding time effects of BM-573 remain unknown. In contrast, ticlopidine, an ADP receptor antagonist, significantly increased bleeding time tendency, as observed by different authors (Kim et al., 1998; Foster et al., 2001). Finally, we examined both the diuretic and hypoglycemic properties of BM-573 compared with torasemide and glibenclamide, respectively. Indeed, BM-573 was originally designed from a pharmacomodulation study of the loop diuretic torasemide that was found to be a weak thromboxane receptor antagonist at a nontherapeutic dosage (Uchida et al., 1992). Thus, the diuretic effects of torasemide and BM-573 were compared in rats over 10 h after intraperitoneal injection of a single dose of 5 mg/kg. As expected, torasemide produced a significant increase in urinary volume over the 10-h period, whereas diuresis induced by BM-573 was not

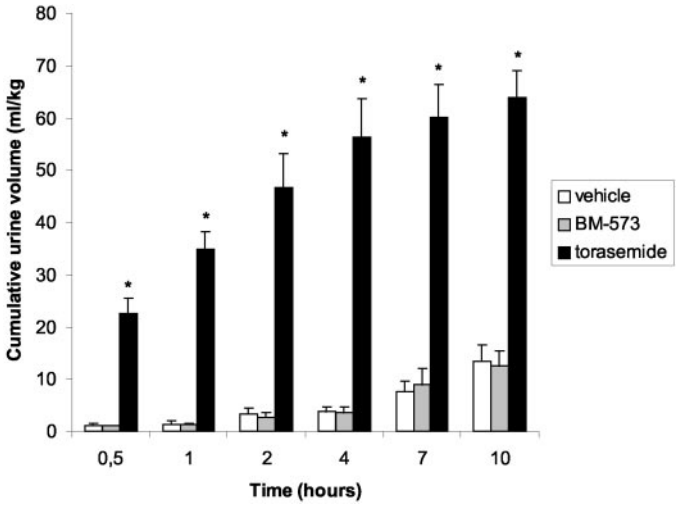



**Fig. 8.** Diuresis (milliters per kilogram) measured in a time-dependent study in rats pretreated by a single dose (5 mg/kg) of BM-573 or torasemide. Columns represent the mean ± S.E.M. of the cumulated urine volumes ($n = 9$). *, $P < 0.05$ compared with the vehicle-treated group.

different from the vehicle-treated group. From a pharmacological point of view, torasemide is a diuretic that acts by inhibiting $Na^+K^+2Cl^-$ cotransport in the thick ascending limb of the loop of Henle. Thus, it can be postulated that BM-573 lost the diuretic property of torasemide because of a lack of affinity for the $Na^+K^+2Cl^-$ cotransport in the thick ascending limb of the loop of Henle (Wittner et al., 1986). This hypothesis can be explained on the basis of the structure-activity studies realized on torasemide derivatives. Indeed, torasemide is a pyridine-3-sulfonylurea derivative. Several studies demonstrated that the sulfonylurea moiety can be substituted by a sulfonylthiourea or a sulfonylcyanoguanidine without dramatically affecting the diuretic properties of torasemide (Masereel et al., 1993). Nevertheless, Wittner and collaborators demonstrated that if the pyridine ring is replaced by a $NO_2$-substituted phenyl ring such as with BM-573, the inhibitory potency for the $Na^+K^+2Cl^-$ cotransport system of the cortical thick ascending limb was lost (Wittner et al., 1987). In vivo experiments performed by our group with other nitrobenzenic sulfonylurea derivatives also confirmed the loss of the diuretic property. The oral

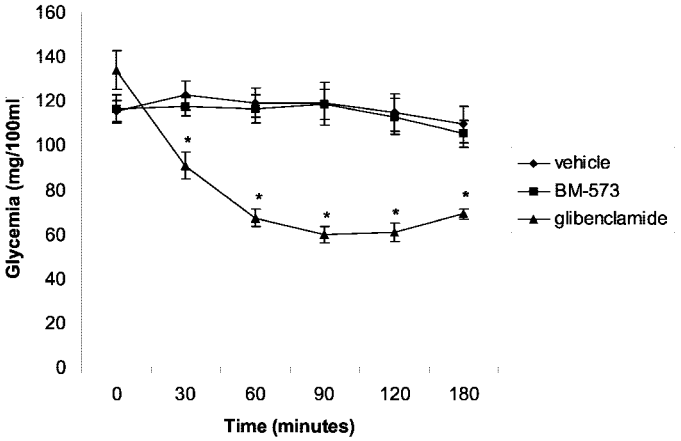



**Fig. 9.** Time courses of the changes in blood glucose level in fed rats injected intraperitoneally at time 0 with BM-573 (20 mg/kg), glibenclamide (20 mg/kg), or the vehicle. Mean ± S.E.M. ($n = 6$). *, $P < 0.05$ compared with the vehicle-treated group.

hypoglycemic glibenclamide, a specific blocker of the ATP-sensitive K+ channel, is the other sulfonylurea derivative that emerged as original TXRA. Indeed, in isolated ring segments of dog coronary artery, glibenclamide has been shown to cause a reduction of both spontaneous isometric force and contractions induced by U-46619 (Cocks et al., 1990; Stanke et al., 1998). Thus, the aim of the last experiment was to evaluate the hypoglycemic property of the sulfonylurea BM-573 compared with glibenclamide in rats. In this test, whereas the hypoglycemic effect of glibenclamide injected in rats was rapid and marked, as observed by Lebrun et al. (2000), BM-573 failed to produce a decrease in blood glucose concentration.

In conclusion, we have demonstrated that the nitrobenzenic sulfonylurea BM-573 is a potent antithrombotic agent that does not affect bleeding time. These effects observed in vivo can be explained by the pharmacological properties of BM-573 at a cellular level. It was indeed characterized as an original combined thromboxane receptor antagonist and thromboxane synthase inhibitor. Moreover, BM-573 lost the diuretic property of torasemide and has no impact on glycemia. The chemical structure of BM-573 offers a new template for the design of original and potent $TXA_2$ modulators useful in thrombotic disorders.

**Acknowledgments**

We thank Patricia Benoit for advice on these experiments and Philippe Neven for excellent technical assistance.

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**Address correspondence to:** Dr. Jean-Michel Dogné, Natural and Synthetic Drug Research Center, University of Liège, Avenue de l'Hôpital, 1, B36, B-4000 Liège, Belgium. E-mail: Jean-Michel.Dogne@ulg.ac.be