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BM-573, a Dual Thromboxane Synthase Inhibitor and Thromboxane Receptor Antagonist Prevents Pig Myocardial Infarction Induced by Coronary Thrombosis^{*}

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BM-573, *N*-terbutyl-*N'*-[2-(4'-methylphenylamino)-5-nitro-benzenesulfonyl] urea;
TXA₂, thromboxane A₂;
LAD, left anterior descending;
LV, left ventricular;
H & E, hematoxylin and eosin;
TTC, 2,3,5-triphenyl tetrazolium chloride;
TS, thromboxane synthase;
AUC, area under the curve;
TnT, troponine T.

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ABSTRACT

The aim of this study was to characterise the effects of BM-573 (*N*-terbutyl-*N'*-[2-(4'-methylphenylamino)-5-nitro-benzenesulfonyl] urea), a novel dual thromboxane A₂ receptor antagonist and thromboxane synthase inhibitor, on myocardial infarction induced by topical ferric chloride (FeCl₃) application to the left anterior descending (LAD) coronary artery in anaesthetised pigs. All control animals (n=6) developed an occlusive thrombus in the LAD coronary artery. The mean infarct size, revealed by triphenyl tetrazolium chloride (TTC) and the area at risk evidenced by Evans blue corresponded to 35.3 ± 2.2 % and 36.9 ± 2.1 % of the left ventricular (LV) mass, respectively. In the BM-573-treated group (n=6), a drug infusion ($10 \text{ mg.kg}^{-1}.\text{h}^{-1}$) started 30 minutes before FeCl₃ application and continued throughout the experimentation. Among the BM-573 treated group, four pigs did not develop coronary artery thrombus and their myocardium appeared healthy. Histopathological examination of FeCl₃-injured coronary artery revealed an occlusive and adherent thrombus in control group while pretreatment with BM-573 prevented thrombus formation. In infarcted zones, lack of desmin staining and muscle structure disorganisation were obvious. Depletion of myocardial ATP content was observed in the myocardial necrotic region of the control group, but not in myocardial samples of BM-573-treated pigs which did not develop myocardial infarction. When BM-573 prevented LAD artery occlusion, the area under the curve (AUC) of plasmatic troponin T was reduced by 77 % over 6h. These data suggest that BM-573 could be useful for the prevention of myocardial infarction.

Cardiovascular pathologies which remain the leading cause of mortality and morbidity in western society include several diseases such as ischemic cardiopathy of which myocardial infarction represents the most important form. Ischemic cardiopathy is characterised by an inadequacy between supply and demand in oxygenated blood correlated with a diminution of coronary blood flow due to coronary artery stenosis or occlusion. This artery occlusion is often caused by atherosclerous lesions, acute thrombosis, oedema, ballooning of atheromatous plaque or bleeding (Pearson et al, 1977; Horie et al, 1978; Koenig, 2001). The consequence of this mismatch is necrosis characterised by the loss of cardiac myocytes (Hillis and Braunwald, 1977). After the onset of myocardial ischemia, it takes 6 hours before myocardial necrosis can be identified by standard microscopic post-mortem examination (Bouchardy and Majno, 1974; Van Reempts et al, 1976; Kloner et al., 1979).

In myocardial ischemia due to plaque rupture or thrombosis, it has been shown that thromboxane A₂ (TXA₂) and other platelet vasoactive mediators are released to promote platelet aggregation and vasoconstriction (Hirsch et al, 1981; Hirsch et al, 1983; Fitzgerald et al, 1986). TXA₂, a short-live lipidic mediator generated by the cyclooxygenase pathway, is mainly produced by platelets, macrophages and lung parenchyma (Hamberg et al., 1975; Moncada and Vane, 1979). TXA₂ is a potent platelet activator and constrictor of vascular and bronchial smooth muscles. It has been demonstrated that drugs able to antagonise TXA₂ receptors or to inhibit thromboxane synthase (TS) reduce the severity of myocardial ischemia (Schorr et al., 1980; Burke et al., 1983; Hock et al., 1986; Brezinsky et al., 1987). Thus, molecules combining a dual activity such as TS inhibition and TXA₂ receptor antagonism have a greater therapeutic interest since they reduce TXA₂ production and prevent the action of TXA₂ and PGH₂ (De Clerck et al., 1989). As torasemide, a loop diuretic (Friedel and Buckley, 1991), showed a weak TXA₂ antagonist activity on dog coronary artery (Uchida et al., 1992), several chemically related molecules were designed and studied for their TXA₂ antagonism

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(Masereel et al., 1999; Dogné et al., 2000; Dogné et al., 2001). Recently, BM-573 (*N*-terbutyl-*N'*-[2-(4'-methylphenylamino)-5-nitro-benzenesulfonyl] urea), a torasemide related molecule (fig. 1), which lost the diuretic properties in rat, showed a high affinity for the human platelet TXA₂ receptor (IC₅₀ : 1.3 nM). BM-573 relaxed the rat aorta contracted by U-46619, a TXA₂ agonist (ED₅₀ : 28.4 nM). Moreover, BM-573 prevented human platelet aggregation induced by arachidonic acid (ED₁₀₀ : 0.13 μM) and completely inhibited TS at 1 μM (Rolin et al., 2001).

In this work, we evaluated the activity of this novel TXA₂ antagonist in a model of myocardial ischemia evoked by thrombosis of the left anterior descending (LAD) coronary artery induced by ferric chloride in pig. To diagnose myocardial infarction, we applied histochemistry of mitochondrial dehydrogenases (Anderson et al, 1979; Fishbein et al., 1981). This method using triphenyl tetrazolium chloride (TTC) allows to reveal and quantify the area of necrosis. TTC stains undamaged myocardium which appears brick red due to the presence of intact dehydrogenase system which reduces TTC in insoluble coloured pigments called formazans (Nachlas and Shnitka, 1963). In necrotic tissue, the dehydrogenase system collapsed, and the area of necrosis is unstained. Samples from infarcted and normal regions were then studied by conventional histological analysis (hematoxylin and eosin, trichrome staining, luxol fast blue) and immunohistochemical identification of desmin, an intermediate filament protein of myocardial muscle cells (Vargas et al., 1999). ATP dosage in normal and infarct regions were also realised for BM-573-treated pigs and compared to the control animals. Troponine T (TnT), a constitutive protein of myofibrils, is a regulator of muscular contraction and an efficient biological marker in myocardial infarction. TnT levels in plasma have been measured to investigate the effect of BM-573 on the increase of TnT due to myocardial infarction.

Methods

Surgical procedure. 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 Guiding Principles for the Care and Use of Laboratory Animals approved by the Council of the American Physiological Society.

Experiments were performed on 18 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⁻¹) and diazepam (1 mg.kg⁻¹). Anesthesia was then induced and maintained by a continuous infusion of sufentanil (0.5 µg.kg⁻¹.h⁻¹) and pentobarbital (5 mg.kg⁻¹.h⁻¹). Spontaneous movements were prevented by pancuronium bromide (0.2 mg.kg⁻¹). After endotracheal intubation through a cervical tracheostomy, the pigs were connected to a volume cycled ventilator (Evita2, Dräger, Lübeck, Germany) set to deliver a tidal volume of 15 mL.kg⁻¹ with a FiO₂ of 0.4 and at a respiratory rate of 20 breaths.min⁻¹. End-tidal CO₂ (ET CO₂) measurements (Capnomac, Datex, Helsinki, Finland) were used to monitor the adequacy of ventilation. Respiratory settings were adjusted to maintain ET CO₂ between 30 and 35 mmHg. Blood pressure and heart rate were measured via a catheter inserted into the ascending aorta. A catheter inserted into the right femoral artery was used for blood sampling. Jugular vein was cannulated for drug administration. Body temperature was monitored and kept at 37°C throughout the experiment using a heating blanket .

The heart was exposed through a median sternotomy. A segment of the LAD coronary artery was isolated distal to the first diagonal branch for thrombus formation. An electromagnetic flow probe (Transonic, Ithaca, NY; USA) was placed at the distal end of the isolated LAD artery to measure the coronary artery blood flow.

Thrombus induction. After a 30 min stabilisation period, a blood sample was drawn (time 0) and a tissue strip (3 mm width) saturated with ferric chloride solution (50 % w/v) was rolled

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around the LAD coronary artery for 45 min. The animals were randomised in three groups: a sham-operated control group (n=6) intravenously infused with BM-573 ($10 \text{ mg.kg}^{-1}.\text{h}^{-1}$), a control group (n=6) intravenously infused with vehicle (propylene glycol-NaCl 0.9 %, 30-70 v/v) and a BM-573-treated group (n=6) infused with BM-573 ($10 \text{ mg.kg}^{-1}.\text{h}^{-1}$). The infusion started 30 min before ferric chloride application and was continuously infused till 6 hours after strip application. When it occurred, the complete occlusion of LAD coronary artery was confirmed by the drop of coronary artery blood flow till zero which appeared within 15-25 minutes.

Quantification of area at risk. The risk area and the infarct area were delineated by a dual staining technique (Higo et al., 1992; Warltier et al., 1975; Toki et al., 1988). Just before the end of the experiment, 20 ml of Evans blue dye solution (0.1 g.ml^{-1} in 50 mM phosphate buffered saline, pH 7.4) was injected into the jugular vein, to stain the non ischemic area blue. The pig was then sacrificed with an injection of potassium chloride. LAD coronary artery was isolated and fixed with 10 % neutral-buffered formaldehyde. The heart was then rapidly removed and sectioned in five transverse slices (0.6 cm thick) from apex to base (S1 to S5). The risk area of left ventricle, due to its anatomical dependence on the LAD coronary artery for blood flow, was identified by lack of the Evans blue in this region. Gross slices have been photographed with a digital camera (Fujifilm FinePix 2400 Zoom).

Quantification of myocardial infarction. Morphological changes in size, shape and transmural distribution of myocardial infarction were measured using 2,3,5 triphenyltetrazolium chloride (TTC) staining (Sigma-Aldrich Chemical Co.). Tissue slices were rinsed with a cold isotonic saline solution and then incubated at 38°C for 15 to 20 min in a phosphate-buffered solution of TTC (1% in 0.1 M, pH 7.4) (Lie et al., 1975; Fishbein et al., 1981). This produced a brick red coloration in the presence of dehydrogenase enzymes in intact myocardium whereas infarcted regions remained unstained due to the collapse of enzyme

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activity. After photography with a digital camera (Fujifilm FinePix 2400 Zoom), slices of myocardium were placed in 10 % neutral-buffered formaldehyde to enhance the contrast between stained and unstained regions. After 3 days of fixation in formalin and just before paraffin proceeding, myocardial sections were still photographed with a video camera (3CCD color video, DXC-390P ExwaveHAD). Infarct size was measured from the tracings of these photographed myocardial slices on to a clear acetate sheet and the area of each zone was then weighted. The ratio between the two zones (ischemic area and nonischemic area) was determined for each slice. Infarct size measured from the tracings of myocardial slices was calculated by planimetry as a percentage of LV mass.

Microscopic analysis. After 3 days, three or four tissue-blocks were taken from each slice at standardised locations and routinely processed for paraffin histology. The tissue blocks from myocardium were cut at 0.6 μ m and each section was stained with hematoxylin and eosin, Masson's trichrome and luxol fast blue. Sections obtained from the artery were stained with hematoxylin and eosin. The stained sections were examined at magnifications of x25, x100, x200 and x400 to study the distribution of infarction. Photomicrographs were taken using a Zeiss photomicroscope (Axioscope 2 plus-Sony 3CCD camera, 1024-768 pixels definition). In addition, all tissue-blocks of slice S3 were processed for immunohistochemical staining of desmin to investigate cardiac muscle's lesions. Monoclonal antibodies to desmin (clone D33, Biomeda) were diluted 1/20.

ATP dosage in myocardial tissue. The ATP content was measured by an adaptation of the ATP bioluminescent assay kit (Sigma-Aldrich diagnostic kit). There is a linear relationship between the relative light intensity generated by luciferin-luciferase reaction and the ATP concentration. Samples of cardiac tissue taken from antero-septal and posterior regions of slice S2 were sequentially frozen in liquid nitrogen and then conserved at -80°C till ATP dosage. Sample was mechanically disrupted, reduced in powder and then suspended in 700 μ l of

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somatic cell ATP releasing reagent. The suspension was then homogenised with a Dounce (10 times), diluted with ultra pure water and 10 μ l added to 100 μ l of ATP assay mix (luciferase, luciferin, MgSO_4 , EDTA, DTT and BSA in a Tricine buffer). For each sample, luminescence in RLU (Relative Light Unit) was determined with a luminometer (LUMAC Biocounter M2010). Bradford's protein dosage was performed, and results expressed in nmol ATP.g^{-1} of protein.

Troponine T dosage in plasma. Arterial blood samples were taken at 0, 3 and 6 h after inducing LAD occlusion with ferric chloride. Collected samples were centrifuged at $2205 \times g$ for 10 min at 15°C . The supernatant was removed and plasma concentration of TnT measured (Lauer et al., 1997). The area under the curve (AUC) is expressed in $\mu\text{g.ml}^{-1}.\text{6h}^{-1}$.

Drugs. BM-573, synthesised in our laboratory, was dissolved in propylene glycol and administered at a concentration of 20 mg/ml.

Statistics. All data are expressed as mean $\pm$ SEM. For analysis of the infarct size, tissue ATP activity and plasma troponine T levels, Student's *t*-test was used. *P* value < 0.01 was considered as statistically significant.

Results

Heart rate and mean aortic blood pressure. Before FeCl₃ application, heart rate (fig. 2a), mean aortic blood pressure (fig. 2b) and their product (fig. 2c) were not affected by infusion of BM-573. While heart rate increased from 110 beats.min⁻¹ to 150 beats.min⁻¹ in control and BM-573-treated group, there was no significant difference in heart rate nor in mean aortic blood pressure nor in their product between both groups throughout the experiment. In the sham-operated control group, BM-573 did not modify heart rate and mean aortic blood pressure throughout the experiment however the starting basal value of blood pressure was higher than that of the other groups (fig 2a) .

Macroscopy and infarct size. The infarct zones were delineated by TTC staining which reveal a collapse of the dehydrogenase system. In the control group (n=6), myocardial infarction is well marked while infarct zones are not present in the BM-573-treated group (n=6) and in the sham-operated control group (n=6). For each control animal, the blood flow of the LAD artery dropped to zero within 25 min, the artery was completely occluded and each pig developed a transmural area of irreversible damage, mainly located in the anteroseptal region of the left ventricle. In the BM-573 treated group, four animals did not develop LAD artery occlusion nor any detectable infarct area as well as in the sham-operated control group. In the BM-573 treated group, two pigs presented a necrotic region due to the LAD artery occlusion (fig. 3). For each pig which developed myocardial infarction, the infarct size, expressed as the percentage of LV mass, was calculated by planimetry from heart slices (S1 to S5). For each slice (S1 to S5), the mean infarct size was similar in control group and in both treated pigs which developed myocardial infarction (table 1.b.). The mean infarct size reported to the LV mass was similar for controls and both BM-573 treated pigs ($35.3 \pm 2.1\%$ and 33.8% , respectively). The risk area has been revealed by Evans blue and then calculated for both

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groups. The mean risk areas calculated for the animals in control group and for the BM-573 treated pigs which developed an infarction were respectively 36.9 ± 2.1 % and 34.8 %. For each animal with occluded LAD artery, this risk area was superimposed to the necrotic zone revealed by TTC staining (table 1.a.).

Histopathological examination of infarcted myocardium. Heart from six control animals was examined by light microscopy. The main ischemic changes were observed in the anterior left ventricle, corresponding to the area supplied by the LAD artery. Pycnosis of nuclei from muscle fibres, oedema and beginning leucodiapedesis from the capillaries suggested that these cells reached the stage of necrosis. If histological diagnosis could be established with hematoxylin and eosin staining, delineation between ischemic and non ischemic region was unclear. For this reason, desmin immunohistochemical staining was used to detect microscopically the necrotic zone. Loss of staining with antibodies to desmin was detected in necrotic myocardium and closely related to the affected area revealed by TTC staining. In healthy regions from control and BM-573 treated pigs, positive immunohistological staining of desmin revealed the Z bands of cardiac muscle cells giving a typical striated appearance (fig. 4a). The pattern and distribution of infarction identified histologically was related to the results obtained by TTC staining (fig. 4b). In the sham-operated group, no histopathological changes were observed.

Histopathological examination of ferric chloride-injured coronary artery. Topical application of ferric chloride on the LAD artery induced an occlusive thrombus adherent to the vessel wall from the site of ferric chloride contact (fig. 5A). Treatment with BM-573 partially prevented thrombus but did not protect against vascular injury due to ferric chloride. The three layers which constitute healthy arterial wall (intima, media and adventice) were damaged at artery application site of ferric chloride (fig. 5B).

ATP dosage in myocardium. ATP content was measured in myocardium from control and BM-573-treated animals. Two samples were judiciously taken from slice n°2 of each heart: one in the antero-septal region and one in the posterior region stained by TTC (fig. 6).

In all control animals with an occlusive thrombus, a significant ($P < 0.01$) myocardial ATP decrease was observed in the necrotic region (11630 ± 3410 vs 76.6 ± 43.6 nmol.g⁻¹ of protein). Compared to the healthy region, a drop of ATP content was also measured in the necrotic area of both BM-573-treated animals which presented LAD coronary artery occlusion (28460 ± 10140 ; 94.1 ± 33.4 nmol.g⁻¹ of protein, respectively, $P < 0.01$). When BM-573 prevented LAD artery occlusion (n=4), ATP contents were similar in healthy and LAD artery supplied zones (9484 ± 2880 ; 16760 ± 4310 nmol.g⁻¹ of protein, respectively) as observed in the sham-operated control group (27590 ± 4781 ; 27140 ± 4721 nmol.g⁻¹ of protein, respectively).

Troponine T levels in plasma. Plasma levels of TnT were measured from control and BM-573-treated animals. A marked elevation of TnT concentration was observed in control group during 6 hours of ischemia while a significantly smaller rise of plasmatic TnT was seen when BM-573 prevented the LAD artery occlusion ($P = 0.0017$). AUC were 2924 ± 278 µg.ml⁻¹.6h⁻¹ and 656 ± 244 µg.ml⁻¹.6h⁻¹, respectively.

Discussion

In previous *in vitro* and *ex vivo* studies, it has been demonstrated that BM-573 was a potent dual compound able to reduce TXA₂ production by TS inhibition and to prevent the action of TXA₂ (or PGH₂) by blocking the TXA₂ receptors (Rolin et al., 2001). The porcine model of myocardial infarction where thrombus formation is induced by ferric chloride placed on the surface of the LAD coronary artery, is a suitable model which produces a mixed thrombus enriched in platelets and fibrin (Kurz et al., 1990).

The present investigation demonstrated that this dual TXA₂ receptor antagonist and TS inhibitor prevents the formation of an occlusive thrombus in the LAD coronary artery without affecting heart rate, mean aortic blood pressure and their product. Indeed, intravenous infusion of BM-573 (10 mg.kg⁻¹.hr⁻¹) 30 min before thrombus induction in the LAD coronary artery prevents any myocardial tissue damage in four treated pigs out of six. As observed for TS inhibitors (Simpson et al., 1987), BM-573 probably prevented the development of myocardial infarction by shunting PGH₂ production to the synthesis of cardioprotective prostacyclin (PGI₂). On the other hand, if competitive TXA₂ receptor antagonists prevent both TXA₂ and PGH₂ binding, they do not increase prostacyclin formation (Gresele et al., 1984). Thus, the combination of these two properties in BM-573 could be considered more beneficial in myocardial ischemic injury than molecules bearing only one of these features : TS inhibitor or TXA₂ receptor antagonist.

The antiaggregant properties of BM-573 (Rolin et al., 2001) prevent the formation of an occlusive thrombus. When platelet aggregation is initiated, this phenomenon is irreversible and

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leads to a complete aggregation (“all or none phenomenon”) (Watanabe et al., 1989; Ruggeri, 1994). This could explain why the LAD artery occluded in two treated pigs.

To evaluate the myocardial protective effects of BM-573, the TTC staining method was used. This technique has been validated and represents a methodology for the early differentiation of infarcted and healthy myocardium. TTC staining can be used 2 hours following coronary occlusion and after reperfusion (Fishbein et al., 1981). This technique relies on reduction of the colourless TTC salt by dehydrogenase enzymes to brightly coloured red compounds in healthy tissue during a rather short incubation. In this study, the risk area was determined by intravenous injection of Evans blue 6 hours after thrombus induction.

Even if similar anatomical sites were chosen to place ferric chloride, the risk area and the infarct size depend on heart size, on coronary anatomy and on development of the coronary collateral circulation. In each pig with an occlusive thrombus, the size of the risk area determined by Evans’ blue staining was similar and superimposed to the infarct zone revealed by TTC. That could be attributed to the pig coronary collateral system less developed than that of human and that is the reason why necrosis progression only occurred after 6-12 hours in man. Since risk area and infarct region are similar, the infarct size was expressed as a percentage of left ventricle. For the six control animals, occlusion of the LAD provoked by ferric chloride at the same anatomical site produced an infarct of 35.3 ± 2.1 % of LV mass. Despite BM-573 infusion, two animals developed a LAD artery occlusion, and their infarct size was of 33.8 % of LV mass.

Histopathological examination revealed myocardial necrosis largely pronounced in vehicle-treated group. This group suffering 6 hours of ischemia showed evidence of the effect of anoxia with several alterations consisting of interstitial oedema, granularity, small leukocytes infiltrates, anisonucleosis, and caryolysis. The infarct was clearly delineated by the lack of TTC staining and there was a close correspondence between gross and histological measurements of

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necrosis area in each slice. Moreover, clear delineation between healthy and ischemic myocardium revealed on gross slices was clearly seen in desmin stained sections and could be superimposed. Positive immunological staining of desmin revealed healthy myocardium with a high organisation level in muscle cells taken from heart of BM-573 treated pigs which did not developed infarction. Each sample taken from the necrotic area showed a negative desmin staining and a loss of organisation of myocardial tissue.

Following ischemia, the drop of ATP content is an indicator of tissue necrosis. A significant ATP depletion was measured for the control group as for both treated pigs in which the LAD artery occluded. When BM-573 prevented LAD artery occlusion, no ATP change was observed and the energetic intermediate level of myocardium was preserved. Plasma dosage of TnT, a sensitive and specific biological marker of necrosis, revealed a decrease of 77 % of the AUC when BM-573 prevented ischemia, as compared to the control group.

In conclusion, we previously showed that BM-573, a ligand of the human platelet TXA_2 receptor (IC_{50} 1.3 nM), was able to relax a rat aorta precontracted with U-46619 (ED_{50} 28.4 nM), to prevent human platelet aggregation induced by arachidonic acid (ED_{100} 0.13 μM) and to completely inhibit TS at 1 μM . Here, we demonstrated that BM-573, a dual TS inhibitor and TXA_2 receptor antagonist was able to prevent the formation of an occlusive thrombus in the LAD artery of BM-573-treated-pigs. They did not develop infarction and their myocardium was totally healthy with a protected structure despite attempt to form thrombus in the coronary artery. The coronary thrombus of each vehicle-treated pig was adherent and occlusive. As expected, BM-573 did not protect against vascular injury due to ferric chloride. These data suggest that BM-573 could be useful in acute coronary syndrome and during coronary angioplasty. Further experiments using an ischemia-reperfusion model will contribute to establish the clinical interest of this dual TS inhibitor and TXA_2 antagonist.

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Footnotes

Footnote to title

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Legend for figures

Fig.1. Chemical structures of torasemide and BM-573

Fig.2. Heart rate (a), mean aortic blood pressure (b), and their product (c) of control (O), BM-573 (■) treated animals and sham-operated control animals (▲). Mean $\pm$ SEM, n=6 for each group.

Fig.3. Photographs of gross slices of hearts after TTC staining from animals treated with vehicle (A), with BM-573 when coronary thrombus formation was prevented (B) and with BM-573 when coronary thrombus formation was not prevented by drug administration (C). Distinct delineation of antero-septal infarction is visible by lack of staining in pictures A and C.

Fig.4. Structure of normal myocardial muscle (a) compared to necrotic myocardium (b) after immunohistochemical detection of desmin from heart of a vehicle-treated pig (x400).

Fig.5. Light micrographs of ferric chloride-induced thrombi from vehicle-treated group (A) and BM-573-treated group (10 mg.kg⁻¹.h⁻¹, iv.) (B). Thrombus is indicated on picture by letter T. Both sections were stained with hematoxylin and eosin (x25).

Fig.6. ATP content (nmol.g⁻¹ of protein) of heart slice n° 2 from control animals (n=6) (A) and BM-573-treated animals without thrombus formation (n=4) (B) (**= $P < 0.01$).

(a) Area at risk

	Vehicle-treated (control)		BM-573-treated (%)
	(%)	(n=6)	(n=2)
S1	81.3	± 4.3	80.6
S2	45.5	± 2.9	57.9
S3	39.2	± 3.1	37.9
S4	30.5	± 3.3	22.8
S5	21.5	± 3.3	15.1
LV	36.9	± 2.1	34.8

(b) Infarcted area

	Vehicle-treated (control)		BM-573-treated (%)
	(%)	(n=6)	(n=2)
S1	71.3	± 4.5	74.5
S2	48.6	± 0.9	49.9
S3	34.5	± 3.5	38.5
S4	27.2	± 1.9	25.1
S5	20.6	± 2.6	15.5
LV	35.3	± 2.1	33.8

TABLE 1. For control animals and both BM-573 treated animals which developed an infarction, the area at risk revealed by Evans' blue (a) and the infarcted area revealed by TTC

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(b) are expressed as the percentage of left ventricle from each slice (S1→S5) and of total left ventricular mass (LV). Four BM-573-treated animals did not develop any myocardial infarction.

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Figure 1

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Figure 2

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Figure 3

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Figure 4

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Figure 5

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Figure 6