Metalloproteinase Inhibition Reduces Constrictive Arterial Remodeling After Balloon Angioplasty

A Study in the Atherosclerotic Yucatan Micropig

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Background—Arterial remodeling after balloon angioplasty has been recognized as a major determinant of restenosis. Perturbation of collagen metabolism might be important. After balloon injury, matrix metalloproteinase (MMP) expression is upregulated. We investigated the effect of Batimastat, a nonspecific MMP inhibitor, on late lumen loss, arterial remodeling, and neointima formation after balloon dilation.

Methods and Results—In atherosclerotic iliac arteries of 12 Yucatan micropigs, balloon dilation was performed, with intravascular ultrasound and quantitative angiography used before and after balloon dilation and at 42-day follow-up. The animals were randomly divided into 2 groups, the Batimastat group (n = 6) and the vehicle group (n = 6). All animals were intraperitoneally injected with either Batimastat or a vehicle immediately after balloon dilation and at 2 weeks and 4 weeks after balloon dilation. Angiographic and echographic late lumen loss in the Batimastat group versus the vehicle group was 0.3 ± 0.1 versus 0.8 ± 0.1 mm (P = 0.01) and 2.2 ± 0.5 versus 4.9 ± 0.7 mm² (P = 0.004), respectively. Late media-bounded area loss was used as a measure of remodeling after balloon dilation and was 0.9 ± 0.6 mm² in the Batimastat group compared with 3.8 ± 0.8 mm² in the vehicle group (P = 0.003, mixed model analysis P = 0.01). Neointima formation was 1.3 ± 0.3 mm² in the Batimastat group and 1.0 ± 0.2 mm² in the vehicle group (P = 0.542).

Conclusions—Metalloproteinase inhibition by Batimastat significantly reduced late lumen loss after balloon angioplasty by inhibition of constrictive arterial remodeling, whereas neointima formation was not inhibited by MMP inhibition. (Circulation. 2000;101:2962-2967.)

Key Words: restenosis □ remodeling □ angioplasty □ balloon

Coronary restenosis after balloon angioplasty has long been considered a result of excessive neointima formation. Recently, however, human and animal studies have shown that arterial circumference can change as well in response to arterial intervention (so-called geometric remodeling) and may explain up to 70% of late lumen loss.

The mechanism of geometric remodeling is still unclear, but initial studies suggest that perturbation of collagen metabolism might be involved. Matrix metalloproteinases (MMPs) are zinc- and calcium-dependent proteases that degrade collagen and other matrix proteins such as elastin and proteoglycans. Recent studies have shown a transient increase in MMP activity after balloon injury. Moreover, overexpression of tissue inhibitor of metalloproteinase-2, which is a natural inhibitor of MMP-1 and MMP-2, delayed aortic rupture in an aneurysm model. We hypothesized that inhibition of MMPs may reduce late lumen loss after balloon dilation by inhibition of constrictive arterial remodeling. It has previously been shown that metalloproteinase inhibitors do not affect intimal hyperplasia after balloon injury. In the present study, we evaluated the effect of Batimastat, a nonspecific MMP inhibitor, on late lumen loss, remodeling, and neointima formation after balloon dilation.

Methods

Twelve 7-month-old Yucatan micropigs were used for the present study. Two weeks after the start of an atherogenic diet, they underwent Fogarty injury of the internal and external iliac arteries. The atherogenic diet was continued for 4 months until selected arteries were balloon-dilated; thereafter, the atherogenic diet was replaced by a regular diet. The results of balloon angioplasty were documented by quantitative angiography and by serial intravascular ultrasound (IVUS). Six weeks later, the animals were euthanized, and arteries were harvested. The study was compliant with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication No. 85-23, revised 1985) and was approved by...
the ethical committee on Animal Experiments, Medical Faculty, Utrecht University.

Atherogenic Diet and Anesthesia

In addition to essential nutrients, 1.5% cholesterol, 17.5% casein, 14% lard, and 6% peanut oil were the atherogenic components of the 2400 kcal/d diet. In previous experiments, this diet resulted in a sustained 10-fold increase in total cholesterol and a 3.5-fold increase in HDL. The diet during follow-up was regular chow with equal daily nutritional value.

For all procedures, the animals were anesthetized with intravenous metomidate (4 mg/kg) and ventilated (Servo, EM 902) with O<sub>2</sub>/N<sub>2</sub>O (1:2) and halothane (1% to 2%). Heparin was used for systemic anticoagulation (Thromboliquine, 100 IU/kg, Organon), and acetylsalicylic acid (125 mg PO) was started 1 day before until 14 days after the intervention. A continuous intravenous infusion of nitroglycerin (20 μg/min) and atropine (0.25 mg IV every 15 minutes) was given.

Procedures

For all procedures, arterial access was obtained through a carotid cutdown and insertion of a sheath. An 8F guiding catheter was advanced to the aortic bifurcation and used for angiography and guidance of balloon and IVUS catheters. After initial injury and intervention, the carotid artery was repaired for future access.

During initial injury, 3- to 4-cm segments (measured by a radiopaque ruler) of the iliac arteries were denuded by triple withdrawal of a 4F Fogarty catheter that was manually inflated with 50/50 (vol/vol) water contrast. Four months after the initial injury, selected sites of angiographic stenosis were randomly assigned to the balloon dilation or control arm in a 4:1 ratio. Before and after each intervention, angiography and IVUS were performed. For balloon dilation, a standard peripheral (length 2 to 4 cm, diameter 4 to 7 mm) balloon catheter was advanced over a 0.035- or 0.014-in guidewire. The balloon was inflated 3 times for 1 minute at 10 atm.

At the 42-day follow-up, repeat angiography and IVUS were performed. The animals were euthanized, and the iliac arteries were isolated, removed, snap-frozen in liquid nitrogen, and stored at −80°C.

Angiography and IVUS

Selective angiography (contrast, Telebrix) was performed before each intervention, after each intervention, and at follow-up by use of a digital C-arm (Philips) at a cine rate of 12/s. The highest contrast image was stored for later analysis.

The angiographic diameters of the arteries were measured by using a semiautomated program as described previously. In each artery, lumen diameters were measured at intervals of 0.5 cm, including the treated segment and proximal and distal reference lumen diameters (RLDs) that were both spaced at least 1 cm away from the treatment site.

The site with minimum lumen diameter (MLD) was determined on angiogram and on IVUS and was used for calculation of, for example, acute gain and late lumen loss. RLD was defined as the average of proximal and distal RLD. Angiographic acute gain was defined as postprocedure minus preprocedure MLD (MLD<sub>post</sub>−MLD<sub>pre</sub>), and late lumen loss was defined as postprocedure minus follow-up procedure MLD (MLD<sub>post</sub>−MLD<sub>fu</sub>). Angiographic stenosis was defined as (1−MLD/RLD)×100. The dilation ratio was calculated as balloon diameter on fluoroscopy divided by RLD.

IVUS recordings were made before intervention, after intervention, and at follow-up by use of a 4.1F 30-MHz transducer (DuMED) at 16 rpm, with an axial resolution of 0.1 mm (Figure 1). IVUS images were analyzed with a digital video analyzer as described previously. To locate the treated segment repeatedly over time (before the procedure, after the procedure, and at follow-up), its position was related to an anatomic landmark and a radiopaque ruler. On the IVUS images, the media-bounded area (MBA) was defined by the area circumscribed by the interface of echoluent media and echodense adventitia. The lumen area was traced, and the minimum lumen area (MLA) was determined. Reference lumen area (RLA) was defined as the average of proximal and distal RLA. Echographic acute gain and late loss were defined as their angiographic analogues and expressed as areas. In addition, late MBA loss, being a measure of remodeling after angioplasty, was defined as postprocedure minus follow-up MBA (MBA<sub>post</sub>−MBA<sub>fu</sub>). Intimal area is MBA minus lumen area. Neointima formation was calculated as the difference between the intima at follow-up and the intima before intervention. Echographic percentage of stenosis was calculated as follows: (1−MLA/RLA)×100.

MMP Inhibition

The nonspecific MMP inhibitor BB-94 (Batimastat) was generously supplied by British Biotech Pharmaceuticals Ltd, Oxford, UK. Batimastat has a peptide backbone that reversibly binds to MMP and a hydroxamic acid group that inactivates catalytic activity. Batimastat inhibits MMP-1, MMP-2, MMP-3, MMP-8, and MMP-9 at nanomolar concentrations.

Animals were randomized to the Batimastat group and the vehicle group. The vehicle was 2.5% ethanol and 2.5% macrogol 400 in 1% (wt/wt) cellulose polymer. Batimastat (64 mg/kg at 20 mg/mL) or

Figure 1. Representative serial IVUS images of atherosclerotic lesion of iliac artery in vehicle group with stenosis of 45% (A, B, and C) and in Batimastat group with an echographic stenosis of 46% (D, E, and F). Before balloon angioplasty (PRE). B and E, immediately after balloon angioplasty (POST). C and F, At 42-day follow-up (FU). M indicates media-bounded area; distance between indicators is 1 mm. Note constrictive arterial remodeling in vehicle-treated artery and absence of remodeling in Batimastat-treated artery.
vehicle was injected intraperitoneally immediately after dilation and at 2 and 4 weeks after balloon dilatation.

**Batimastat Measurements and MMP Activity**

EDTA plasma samples were taken before injection and 1 and 2 weeks after the first injection of Batimastat and stored at −80°C until assay. Plasma Batimastat levels were measured by spiking samples with recombinant MMP-7. MMP-7 activity was measured by using an internally quenched fluorescent peptide assay.¹⁹ Batimastat levels were calculated by comparing MMP-7 activity in the sample with standard calibration curves of Batimastat constructed at the same dilutions as the samples.

Two additional Yucatan micropigs were used to measure MMP activity in the arterial wall 4 days after balloon dilatation at the anticipated peak of MMP activity. To prevent dissociation of Batimastat from metalloproteinases, arterial samples were freeze-dried, pulverized, and resuspended in MMP assay buffer (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 5 mmol/L CaCl₂, 1 μmol/L ZnCl₂, and 0.01% [vol/vol] Brij-35) at 4°C. After centrifugation at 13 000 rpm, a chromogenic MMP activity assay was performed on the supernatant, as previously described.²⁰ In brief, various amounts of active MMP-9 (Fuji) were preincubated with tissue extract for 1 hour, and the total MMP-9 fraction was captured by use of a MMP-9 antibody–coated plate. The antibody recognizes active and inactive human and porcine MMP-9 as well as MMP-9 complexed with tissue inhibitors of metalloproteinases.²¹ MMP-9 activity was then determined in an assay buffer to which 5 μg/mL modified pro-urokinase (U/Kcol, TNO Prevention and Health) and 0.4 mmol/L chromogenic substrate pyro-Glu-Gly-Arg-p-nitroanilide (Chromogenix) were added. Purokinase was modified by substituting Pro-Arg-Phe-Lys at positions 155 to 158 for Arg-Pro-Leu-Gly to create a Gly-Ileu added. Purokinase was modified by substituting Pro-Arg-Phe-Lys

**MMP Inhibition and Late Lumen Loss, Remodeling, and Neointima Formation**

Batimastat caused >50% reduction of late lumen loss as measured by angiography and IVUS (Figure 2). Late lumen loss was 0.3±0.1 mm in the Batimastat group and...
Angiographic and Echographic Parameters

<table>
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<th>Batimastat</th>
<th>Control</th>
<th>Batimastat</th>
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<tr>
<td>MLA_{PRE}, mm$^2$</td>
<td>9.3±0.8</td>
<td>7.5±0.7</td>
<td>9.9±0.6</td>
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<td>MBA_{PRE}, mm$^2$</td>
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<td>9.9±1.0</td>
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<td>MLA_{POST}, mm$^2$</td>
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<td>MBA_{POST}, mm$^2$</td>
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<td>17.1±0.9</td>
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<td>MLA_{FU}, mm$^2$</td>
<td>10.6±0.7</td>
<td>7.6±1.0</td>
<td>9.3±0.7</td>
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<td>MBA_{FU}, mm$^2$</td>
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<td>9.7±1.2</td>
<td>13.3±0.9</td>
<td>10.5±1.8</td>
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<td>MLA_{DE}, mm</td>
<td>2.6±0.1*</td>
<td>2.4±0.3</td>
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<tr>
<td>MLA_{POST}, mm</td>
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<td>MLA_{FU}, mm</td>
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<td>2.5±0.3</td>
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<td>Stenosis by IVUS, %</td>
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<td>22.0±6.3</td>
<td>23.1±4.7</td>
<td>18.6±5.6</td>
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<tr>
<td>Neointima formation, mm$^2$</td>
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<td>−0.4±0.4</td>
<td>1.0±0.2</td>
<td>−0.2±0.1</td>
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Data are mean±SEM. PRE indicates before balloon dilation; POST, immediately after balloon dilation; FU, 42-day follow-up; and Angio, angiography.

*P<0.05 for balloon-dilated arteries from Batimastat group vs vehicle group.

Figure 2. Late lumen loss (LA loss), late MBA loss (MBA loss), and intimal gain (IH gain) at 42-day follow-up after balloon angioplasty for Batimastat group (solid bars) and vehicle group (open bars), assessed by IVUS. *P=0.004 for Batimastat vs vehicle group.

Figure 3. Macrophages in arterial wall of balloon-dilated arteries of animals treated with vehicle (open bars) or Batimastat (solid bars). Intima and adventitia had largest number of macrophages, whereas media had almost none. No differences were observed between vehicle- and Batimastat-treated arteries.

0.8±0.1 mm$^2$ in the vehicle group (P=0.01). Echographic late loss was also smaller in the Batimastat group: 2.2±0.5 mm$^2$ versus 4.9±0.7 mm$^2$ in the vehicle group (P=0.004). Late MBA loss was used as a measure of remodeling after balloon dilation and was 0.9±0.6 mm$^2$ in the Batimastat group compared with 3.8±0.8 mm$^2$ in the vehicle group (P=0.003). Because we had up to 4 data points (arteries) of MBA loss per pig, from internal and external iliac arteries on the left and right side, we further analyzed the effect of treatment while accounting for artery type and artery side. From this mixed model of variance, no interaction of artery type or artery side with treatment was observed (both P>0.25). Neither the effect of artery side nor the effect of artery type on MBA loss was statistically significant (both P>0.50). The effect of treatment, however, remained statistically significant (P=0.01). Neointima formation was unchanged: 1.3±0.3 mm$^2$ in the Batimastat group and 1.0±0.2 mm$^2$ in the vehicle group (not significant at P=0.542). Angiographic and echographic net gains were small in these mildly stenotic arteries, but they were significantly different between the groups. Angiographic net gain was 0.2±0.1 mm in the Batimastat group versus −0.2±0.1 mm in the vehicle group (P=0.01). Likewise, echographic net gain was 1.3±0.5 mm$^2$ in the Batimastat group versus −0.6±0.5 mm$^2$ in the vehicle group (P=0.01).

Immunohistochemistry

To address the potential effect of Batimastat on cell migration and inflammation, we counted macrophages in the adventitia, media, and adventitia of external iliac arteries from the vehicle (n=9) and the Batimastat (n=9) group. As expected, most macrophages were found in the adventitia and the intima, and relatively few were found in the media (Figure 3). No differences were observed in the number of macrophages in the adventitia (vehicle, 39±18 macrophages; Batimastat,
Discussion

The principal finding of the present study was that MMP inhibition by Batimastat reduced late lumen loss after balloon angioplasty by the reduction of constrictive arterial remodeling. Arterial remodeling after balloon angioplasty has been recognized as a major determinant of restenosis. Although balloon-dilated arteries are capable of the whole range of remodeling from enlargement to shrinkage, the latter seems to be the predominant form in animal models and patients with restenosis. Therefore, postdilation remodeling in concert with neointima formation usually leads to a reduction in lumen size.1-5 Furthermore, in a previous study, we found that late lumen loss correlated strongly with late MBA loss (r=0.92) but only weakly with neointima formation (r=0.46), suggesting that geometric remodeling is a more important determinant of restenosis. This has been confirmed for human coronary arteries.23

Several studies have shown that MMP expression is upregulated after balloon injury of rat carotid arteries,12,14 rabbit iliac arteries,7 and pig coronary arteries,15 and it has been suggested that MMPs play an important role in vascular tissue remodeling and neointima formation after balloon dilation through the inhibition of smooth muscle cell proliferation or migration.7,12 In this serial angiographic and IVUS study in the atherosclerotic Yucatan micropig, MMP inhibition resulted in a 71% reduction of remodeling and a 50% reduction of late lumen loss, whereas it had no effect on neointima formation. Both in vitro and in vivo, MMP inhibition had no effect on smooth muscle cell proliferation,7,24 but MMP inhibition did suppress smooth muscle cell migration in vivo14 and in vitro.24-25 However, data on the effect of MMP inhibition on neointima formation in vivo are inconclusive. Although a reduction of neointima formation was observed with Batimastat14 and GM6001,12 there was a catch-up of neointimal growth in the second week after dilation, partially or completely negating the initial reduction. In the present study, in atherosclerotic arteries with preexistent plaque, neointima formation was not inhibited by Batimastat. An effect of Batimastat on cell migration was also unlikely because we observed no differences in the distribution of macrophages in the arterial walls of vehicle- and Batimastat-treated animals. Collectively, these studies indicate that nonselective metalloproteinase inhibition does not affect neointima formation.

Batimastat inhibited MMP activity at the tissue level, although the reduction of metalloproteinase activity was modest with respect to endogenous inhibitory activity. Local MMP inhibition presumably leads to a reduction in collagen and matrix breakdown. How this affects renarrowing of the artery after balloon angioplasty or, more specifically, remodeling is not clear. A study with the metalloproteinase inhibitor GM6001 showed that collagen degradation was inhibited but that at the same time collagen synthesis was inhibited as well, resulting in a net decrease in collagen content of the arterial wall.7 The decrease in collagen content after metalloproteinase inhibition is in contrast with an increase in collagen content after balloon dilation.7,26,27 It is likely that the shape and size of the rigid collagen cylinder determines the lumen size of the artery rather than the gross amount of collagen in the arterial wall. Therefore, we hypothesize that the observed reduction in constrictive arterial remodeling and late lumen loss by Batimastat in the present study is due to inhibition of the breakdown of the “old” extracellular matrix skeleton of the artery, thus preventing shrinkage after balloon angioplasty.

The MLD before balloon dilation was smaller in the Batimastat group than in the vehicle group. However, the dilation ratio and angiographic and echographic acute gain, which are considered to reflect the severity of injury imparted on the arterial wall, were similar in both groups. Therefore, it is unlikely that the difference in MLD before balloon dilation...
has influenced the results. Although the arteries contained considerable preexisting plaques, the angiographic and echographic stenoses were moderate. The relatively small net gain that we observed in the present study is explained by these moderate stenoses and by using clinically relevant dilation ratios of 1.2 and not overdistending the arteries.

In summary, we have shown that MMP inhibition by Batimastat significantly reduced late lumen loss after balloon angioplasty by inhibition of constrictive arterial remodeling and not by inhibition of neointima formation. We have shown that Batimastat exerts its effect most likely by reducing tissue MMP activity. These findings, together with the strong correlation between remodeling and late lumen loss found in animal5 and clinical6 studies, suggest that for the prevention of restenosis after balloon angioplasty, more benefit can be achieved by inhibiting tissue MMP activity than by inhibiting neointima formation. If the results with an MMP inhibitor can be reproduced in human coronary arteries, constrictive arterial remodeling after balloon angioplasty might be prevented.

Acknowledgments
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References
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