Oral Matrix Metalloproteinase Inhibition and Arterial Remodeling After Balloon Dilation
An Intravascular Ultrasound Study in the Pig

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Background—Inhibition of matrix metalloproteinase (MMP) activity after balloon angioplasty by intraperitoneal injection of batimastat reduces late lumen loss by inhibition of constrictive remodeling. In the present study, we investigated whether the oral MMP inhibitor marimastat inhibits constrictive remodeling in favor of neutral or expansive remodeling.

Methods and Results—In 26 pigs, balloon dilation was performed in 101 peripheral arteries. Pigs were treated with marimastat or served as controls and were euthanized 42 days after intervention. Intravascular ultrasound was performed at all time points. Vessel area (VA) loss was assessed by calculating the change in VA at termination relative to after intervention. Arteries were divided in 3 categories: expansive remodeling (VA loss < −5%), neutral (−5% ≤ VA loss ≤ +5%), and constrictive remodeling (VA loss > +5%). In the marimastat group, a significant reduction (53%) of late lumen loss was observed that was fully explained by impaired constrictive remodeling. In the marimastat group, the prevalence of constrictive remodeling was reduced (38% versus 75% in the control group) in favor of not only neutral but also expansive remodeling (21% and 42% versus 4% and 21% in the control group, respectively, P < 0.01). In contrast to the control group, acute luminal gain in the marimastat group did not correlate with late VA loss.

Conclusions—Irrespective of the acute luminal gain by balloon dilation, the oral MMP inhibitor marimastat inhibited constrictive arterial remodeling in favor of both neutral and expansive remodeling. (Circulation. 2001;103:302-307.)

Key Words: metalloproteinases ■ angioplasty ■ stenosis ■ remodeling ■ ultrasonics

For a long time, the 30% to 50% angiographic restenosis rate after balloon angioplasty has been attributed to excessive neointima formation. More recently, both human1,2 and animal3–6 studies have revealed that constrictive arterial remodeling is the major determinant of restenosis after balloon angioplasty. Arterial remodeling in this context means a structural change in total circumference at the dilated site that ranges from expansive to constrictive remodeling (shrinkage).

Collagen is the major determinant of arterial stiffness and may be considered to be the skeleton of the artery. It has been demonstrated that enhanced collagen breakdown and synthesis are important features in arterial remodeling in the first weeks after balloon angioplasty.7–9 Collagen can be degraded only by a family of matrix metalloproteinases (MMPs) that belong to a group of zinc- and calcium-dependent proteases. After balloon angioplasty, a transient increase in MMP activity has been observed.8

Recently, we reported that intraperitoneal injection of batimastat, a nonspecific MMP inhibitor, after balloon angioplasty in atherosclerotic Yucatan minipigs resulted in a significant (50%) reduction in late lumen area loss (LLL) by inhibition of constrictive remodeling.10 Neointima formation was not inhibited.

Little is known about the underlying mechanisms of MMP inhibition after balloon angioplasty. It is unknown whether expansive remodeling is blocked by MMP inhibition as well, resulting in “neutral” remodeling, eg, no change in total circumference. Furthermore, the effect of MMP inhibition on the relationship between acute luminal gain and the 2 determinants of restenosis, arterial remodeling and intimal hyperplasia, is unknown.

In the present study, we monitored the effect of the nonspecific MMP inhibitor marimastat (BB-2516) after balloon dilation in the pig. Marimastat is orally administered, which is a major advantage over batimastat (BB-94), which has to be administered intraperitoneally. It is being applied clinically to prove its role as a tumoristatic agent.11 Marimastat has a collagen-like backbone, which facilitates binding to...
the active site of the MMP, and a hydroxymate group, which
chelates the zinc ion in the active site. All known classes of
MMPs in the nanomolar range are inhibited. An unpublished
dosimetry study in Landrace pigs in our laboratory revealed
that a dose of 10 mg/kg twice a day would give an exposure
of 100 to 200 ng/mL plasma, a concentration that has
previously been effective in animal models of cancer.

With intravascular ultrasound (IVUS), arterial remodeling
and intimal hyperplasia were assessed with and without MMP
inhibition and related to short-term luminal gain. Further-
more, we investigated whether MMP inhibition results in
enhanced expansive remodeling or in blocking of constrictive
remodeling in favor of only neutral remodeling.

Methods

Animals

Twenty-six nonatherosclerotic Landrace pigs with an average weight
of 20 kg were studied. Balloon dilation was performed in 50 femoral
and 51 internal iliac arteries. The animals were euthanized 42 days
after intervention. Angiography and IVUS were performed at all
time points. The investigation was approved by the Ethical Commit-
tee on Animal Experimentation of the Faculty of Medicine, Utrecht
University.

Anesthesia

During intervention and euthanization, the animals were anesthetized
with intravenous midazolam 0.3 mg · kg⁻¹ · h⁻¹ and sufentanil 2.5
µg · kg⁻¹ · h⁻¹ and ventilated with a mixture of O₂ and air, 1:1, and
halothane 1% after a premedication with azaperone 4 mg/kg.

Intervention

Acetylsalicylic acid was administered to all pigs (80 mg/d), starting
with 320 mg 1 day before the intervention. Animals were heparin-
ized (100 IU/kg), and a continuous infusion of nitroglycerin (20
µg · kg⁻¹ · min⁻¹) was given to prevent arterial spasm. The arterial tree
was accessed through a right carotid approach. An arterial 8F sheath
was introduced into the descending aorta. An 8F guiding catheter was
advanced to the aortic bifurcation. Through the guiding catheter,
contrast angiography was performed and the IVUS catheter was
advanced. For balloon dilation, a standard peripheral balloon catheter
(length 2 to 4 cm, diameter 4 to 6 mm) was advanced to a location
that was identical for all pigs. Pigs underwent balloon dilation of the
femoral and internal iliac arteries at both sides. With a balloon/artery
ratio of 1.2, the balloon was inflated 3 times for 1 minute at a
pressure of 8 to 10 atm. After intervention, the right carotid artery
pressure of 8 to 10 atm. After intervention, the right carotid artery
was ligated. For adequate pain relief, buprenorphine hydrochloride
was used for correction of growth of all cross-sectional areas as follows:

\[
\text{VA loss} = \frac{\text{mean } r^2 \text{ at death} - \text{mean } r^2 \text{ at } \text{ intervention}}{\text{mean } r^2 \text{ at death}}
\]

The radius of the untreated segments (r) was determined
with the angiogram and confirmed by the existence of acute
death. The radius of the untreated segments (r) was determined
during manual pullback and at regular intervals with the IVUS
catheter held still.

Termination

After angiography and IVUS, the animals were killed by an overdose
of pentobarbital.

MMP Inhibition

Marimastat (BB-2316) was supplied by British Biotech Pharma-
caceuticals Limited. The animals were randomly divided into a control
group and a marimastat group. The marimastat group was subdivided
into 3 groups with different periods of marimastat administration (2,
4, and 6 weeks, respectively) for each group and for the purpose of another study. The
follow-up of each group, including the control group, was 6 weeks.
The animals started with marimastat, 10 mg/kg twice a day, 1 day
before the intervention. Neither the toxicities described in previous
animal studies (data on file, British Biotech, Inc) nor the adverse
events of marimastat reported in human clinical trials were ob-
erved in the marimastat-treated pigs.

Marimastat Measurements at Tissue Level

Functional marimastat was detected in vascular extracts as follows.
To 10 µL of vascular extract, 1 ng/mL of a tight-binding MMP
inhibitor (BB94) and 45 ng/mL active MMP-9 were added and
incubated at 37°C for 20 minutes. Subsequently, the mixture was
incubated overnight at 4°C on plates coated with anti–MMP-9
antibody (Amersham Pharmacia Biotech RPN 2630 MMP-9 activity
assay kit). After capture, the plate was washed with buffer, and
bound MMP-9 activity was determined according to the kit instruc-
tions. The measured activity originates from the MMP-9/marima-
s tat complex in the vessel sample and is a representation of the
effective marimastat concentration in the extract. Results were
quantified by comparison with a reference line of various marimastat
concentrations (0 to 100 ng/mL) in untreated control vessel extracts.
The detection limit of the method is ~1 ng/mL added marimastat.

Data Analysis

Untreated segments (proximal parts of external iliac arteries, parts
proximal and distal to the dilated segments of the femoral arteries,
parts distal to the dilated segments of the internal iliac arteries) were
used for correction of growth of all cross-sectional areas as follows:

\[
1 - \left( \frac{\text{mean } r^2 \text{ at death} - \text{mean } r^2 \text{ before intervention}}{\text{mean } r^2 \text{ at death}} \right)
\]

The radius of the untreated segments (r) was determined
graphically.

The IVUS images were analyzed at regular intervals (every 0.5
cm). Anatomic landmarks were used to match the images at different
time points. The balloon-dilated segment was identified by compar-
ison with the angiogram and confirmed by the existence of acute
luminal gain, which was defined as the difference between postint-
erven tion and preintervention lumen areas. Vessels lacking proce-
dural gain (gain ≤ 0 mm) were excluded from further analysis.
Within the balloon-dilated segment, the location with the smallest
lumen area at follow-up was selected for further calculations. LLL
was defined as the difference between lumen area after intervention
and at follow-up. Vessel area (VA) before and after intervention was
defined as the inner border of the echolucent layer within the IVUS
image and therefore equals lumen area before and after intervention.
At death, the VA was defined as the outer border of the echolucent
layer and therefore represents lumen, intimal, and medial area. Loss
in VA, being a measure of remodeling, was calculated by subtracting
the VA after intervention and at follow-up. Intimal hyperplasia was
calculated by the difference between VA and lumen area at follow-
up. For each location, the mode of remodeling (constrictive, neutral,
or expansive) was assessed by calculating the VA at follow-up
relative to VA after intervention. The arteries were divided into 3
categories: expansive remodeling (VA loss < −5%), neutral remodel-
ing (−5% ≤ VA loss ≤ +5%), and constrictive remodeling (VA
loss > +5%).
Statistical Analysis
SPSS 8.0 was used for all statistical calculations. An independent-sample \( t \) test was used for differences between mean values, including maximal and minimal mean values among the different marimastat groups. A \( \chi^2 \) test was used for differences in distribution of VA changes among groups. A value of \( P < 0.05 \) was considered to be statistically significant. Gain versus LLL and gain versus late VA loss relationships were calculated with linear regression.

Results
Balloon dilation was performed in 24, 21, and 24 arteries of pigs receiving marimastat for 2, 4, and 6 weeks, respectively (\( n=6 \) pigs for each group). In the control group, balloon dilation was performed in 32 arteries of 8 pigs. Nine, 5, and 4 arteries in the marimastat groups and 4 arteries in the control group were excluded because of either lack of gain, extravasation, or excessive thrombus formation (thrombus occupying \( >30\% \) of lumen area). One pig that was supposed to receive marimastat for 6 weeks (4 arteries) and 1 control pig (4 arteries) died unexpectedly because of ventricular fibrillation and because of a retroperitoneal bleeding due to the intervention, respectively.

In vessel extracts of marimastat-treated animals, 1 to 10 ng/mL functional marimastat was detected.

The Table lists the IVUS measurements at different time points. The different marimastat groups have been pooled, because the duration of administration did not affect the outcome of marimastat treatment (see Table, \( P > 0.1 \) for LLL, VA loss, and intimal hyperplasia). Acute luminal gain did not differ significantly between the 2 groups: 4.56 ± 3.03 mm\(^2\) in the marimastat group versus 4.92 ± 3.64 mm\(^2\) in the control group (\( P = 0.68 \)).

**Marimastat and LLL, Late VA Loss, and Intimal Hyperplasia**
Figure 1 shows the effect of marimastat on LLL, late VA loss, and intimal hyperplasia. A 53\% reduction in LLL was observed in the marimastat-treated group compared with the control group. In the marimastat group, LLL was 2.51 ± 2.61 mm\(^2\) versus 5.31 ± 4.78 mm\(^2\) in the control group (\( P = 0.01 \)). Late VA loss was −0.24 ± 2.85 mm\(^2\) in the marimastat group versus 3.02 ± 4.48 mm\(^2\) in the control group (\( P < 0.01 \)). In the marimastat group, the reduction in LLL was completely due to the reduction in late VA loss; no significant difference in intimal hyperplasia was observed between the 2 groups: 2.74 ± 1.88 mm\(^2\) in the marimastat group versus 2.29 ± 1.54 mm\(^2\) in the control group (\( P = 0.28 \)).

VA at Follow-Up Compared With After Intervention
Figure 2 shows the distribution of the VA loss in both groups. Compared with the control group, marimastat inhibited constrictive remodeling in favor of both neutral and expansive remodeling (\( P < 0.01 \)).

Acute Gain and Late VA Loss
Figure 3 shows the relationship between acute gain and late VA loss in the control group and marimastat group. In the control group, a significant correlation was observed. In the marimastat group, however, this correlation was lost.

Acute Gain and Intimal Hyperplasia
Figure 4 shows the relationship between acute gain and intimal hyperplasia in the control group and marimastat group. In both groups, the impact of luminal gain on intimal hyperplasia was small but significant.

Marimastat and VA Growth
In untreated segments, the increase in VA did not differ among groups (\( P = 0.90 \)). During follow-up, the mean VA growth of untreated segments was 18.66\% (based on 16 pigs)
in the marimastat group versus 19.33% in the control group (7 pigs).

**Discussion**

The principal findings of the present study were that oral MMP inhibition by marimastat reduced LLL after balloon dilation by blocking constrictive remodeling in favor of both neutral and expansive remodeling. Intimal hyperplasia was not inhibited. In addition, marimastat blocked the constrictive remodeling response irrespective of the acute luminal gain.

**Marimastat and Constrictive Remodeling**

Constrictive remodeling is the major determinant of restenosis after balloon angioplasty. Breakdown and buildup of the extracellular matrix is likely to occur during this process, which might be comparable to wound contraction. Geary et al. showed in the atherosclerotic monkey that the pattern of matrix and integrin expression within the injured wall is in many ways analogous to that of healing wounds. In an in vitro wound contraction model in which fibroblasts are grown in a collagen matrix, marimastat inhibits lattice contraction mediated by fibroblasts, a modeling that may happen when granulation tissue contracts in a healing wound. These results emphasize the role of MMPs in arterial wall healing after interventional injury.

The present study supports our earlier observations of the potential therapeutic effect of MMP inhibition by batimastat. Marimastat is administered orally, which is a major advantage over batimastat, and has already been clinically applied as a tumoristatic agent.
Marimastat and Intimal Hyperplasia

Several studies support the role of MMPs in smooth muscle cell (SMC) migration to the intima. Antibodies to 72-kD type IV collagenase, as well as batimastat, inhibit SMC migration in vitro. Zempo et al demonstrated suppression of intimal thickening after arterial injury in the rat by batimastat. This reduction of intimal area, however, was less at 14 days than at 7 days.

Neointima formation was not inhibited in the present study or in the previous study with batimastat. Bendek et al demonstrated a significant reduction in SMC migration in MMP inhibitor–treated rats, resulting in a significant decrease in lesion size early after injury. However, prolonged SMC replication in the MMP inhibitor group resulted in lesion size catching up to controls, resulting in the same intimal area 14 days after balloon injury. It is likely that this “catch-up” phenomenon occurred in the present study, as well as in the study of De Smet et al, considering the 42-day follow-up period.

Marimastat and Expansive Remodeling

Marimastat blocked constrictive remodeling not only in favor of neutral remodeling but also in favor of expansive remodeling. A possible explanation for this effect of marimastat is that MMPs are involved in the solubilization of plasma membrane receptors, such as tumor necrosis factor-α receptors, through proteolytic cleavage of the ligand-binding domain at the cell surface. This process, called “shedding,” is an important mechanism for regulating cytokine function and receptor signaling and might be different in constrictive compared with expansive remodeling. Hence, marimastat may affect each mode of remodeling in a different way. Furthermore, MMPs may be involved in the maturation of collagen, because propeptides of collagen are cleaved by MMPs. MMP inhibition might result in the production of less mature collagen, which might lead to more mechanical expansion of the vessel wall. However, Spears et al observed that inhibition of collagen maturation by inhibition of collagen cross-linking did not induce expansive remodeling, which would contradict this hypothesis.

The question arises whether MMP inhibition will enhance aneurysm formation. This seems unlikely, because batimastat limits the expansion of experimentally created abdominal aortic aneurysms. Furthermore, it has been demonstrated that marimastat inhibits elastin degradation in a model of aneurysm disease. MMP inhibition by RS 132908 suppresses aneurysmal dilation in the elastase-induced rodent model of abdominal aortic aneurysm.

Marimastat and Acute Gain: The Bigger, the Better

Maximizing the postangioplasty vessel diameter by deeper, more severe injury might be counterproductive over time because of augmented neointima formation and constrictive remodeling. In the rabbit artery, however, Van Erven et al found no difference in intimal hyperplasia within a wide range of arterial injury. In the present study in the pig, the impact of acute luminal gain on intimal hyperplasia was relatively small in both groups. In addition, no relation between acute luminal gain and late VA loss was observed in the marimastat-treated group, indicating that marimastat blocked constrictive remodeling irrespective of the acute luminal gain. Thus, in our animal model, the motto “the bigger, the better” may apply to balloon dilation in MMP-inhibited arteries. One should realize, however, that this motto cannot be applied to the increased complication rate with larger balloons, which may not be reduced by marimastat treatment.

Limitations of the Study

Constrictive and expansive remodeling after balloon angioplasty have been reported extensively in different animal models. The time frame over which constrictive remodeling develops, however, differs between animals and humans. In peripheral arteries of the atherosclerotic pig, constrictive remodeling starts within days after balloon angioplasty and seems to be maximal at 6 weeks. In humans, the coronary artery shows expansive remodeling in the first month, whereas constrictive remodeling is observed between 1 and 6 months after the intervention.

In the present study, balloon dilation was performed in internal iliac and femoral arteries. These arteries might respond differently to balloon dilation than do coronary arteries.

The present study was performed in a nonatherosclerotic model. It is likely that marimastat will exert antirestenotic effects in an atherosclerotic model as well, because in the atherosclerotic pig model, the precursor of marimastat, batimastat, reduced LLL also by ~50%. One must keep in mind, however, that in both models, balloon dilation resulted in an overdilation of the artery, which could result in reduced local shear force. This is known to induce MMP activity and might also be influenced by MMP inhibition.

Conclusions

The oral MMP inhibitor marimastat inhibited constrictive arterial remodeling after balloon dilation in favor of both neutral and expansive remodeling and irrespective of acute luminal gain. If these results can be reproduced in human coronary arteries, MMP inhibition by marimastat may effectively improve the long-term outcome of coronary balloon angioplasty.

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