MRI of Coronary Wall Remodeling in a Swine Model of Coronary Injury
using an Elastin-Binding Contrast Agent

Running Title: von Bary et al: MRI of Coronary Wall Using an Elastin Specific Compound

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Abstract

**Background**—The extracellular matrix (ECM) plays an important role in the pathogenesis of atherosclerosis and in-stent-restenosis. Elastin is an essential component of the ECM. ECM degradation can lead to plaque destabilization while enhanced synthesis typically leads to vessel wall remodeling resulting in arterial stenosis or in-stent-restenosis following stent implantation. The objective of this study was to demonstrate the feasibility of magnetic-resonance-imaging (MRI) of vascular remodeling using a novel elastin-binding contrast agent (BMS-753951).

**Methods and Results**—Coronary injury was induced in 6 pigs by endothelial denudation and stent placement. At day 28, delayed enhancement (DE-MRI) coronary-vessel-wall-imaging was performed pre and post injection of Gd-DTPA. Two days later, DE-MRI was repeated after administration of BMS-753951. Contrast-to-noise-ratio (CNR) and areas of enhancement were determined. DE-MRI with BMS-753951 caused strong enhancement of the aortic, pulmonary artery and injured coronary artery walls while Gd-DTPA did not. DE-MRI of the stented coronary artery with BMS-753951 yielded a 3-fold higher CNR when compared to the balloon injured and control coronary artery (21±6 vs. 7±3 vs. 6±4; p<0.001). The area of enhancement correlated well with the area of remodeling obtained from histological data ($R^2 = 0.86$, p < 0.05).

**Conclusions**—We demonstrate the non-invasive detection and quantification of vascular remodeling in an animal model of coronary-vessel-wall-injury using an elastin-specific MR contrast agent. This novel approach may be useful for the assessment of coronary-vessel-wall-remodeling in patients with suspected coronary artery disease. Further studies in atherosclerotic animal models and degenerative ECM disease are now warranted.

**Key Words**: MRI, remodeling, extracellular matrix, neointima formation, vascular injury
Extracellular matrix (ECM) synthesis and degradation plays an important role in the initiation, progression and complication of atherosclerosis, in-stent-restenosis as well as in aneurysm formation and degradation or graft disease. Advanced ECM degradation can lead to plaque instability and subsequent plaque rupture as typically observed in patients with acute coronary syndrome. In contrast, enhanced synthesis of ECM typically leads to expansive or constrictive vessel wall remodeling resulting in plaque stability, but also arterial stenosis or in-stent restenosis following stent implantation. Collagen and elastin are important components of ECM. Elastin stabilizes the arterial structure by inducing a quiescent contractile state in vascular smooth muscle cells and its content is significantly increased following endoluminal vessel wall injury, which closely correlates with remodeling of the arterial wall. Upregulation of elastin has also been demonstrated in autopsy samples from patients with carotid atherosclerosis and aortic aneurysms. Hence, an elastin-specific imaging agent may facilitate non-invasive detection of arterial remodeling.

Cardiovascular magnetic resonance (CMRI) imaging for noninvasive in vivo visualization of the coronary artery vessel wall has been successfully implemented in humans with and without the aid of contrast agents. These implementations, however, were limited by the use of non-specific compounds, which rapidly extravasated into the vessel wall and enhanced areas with either increased distribution volume and delayed clearance (typically fibrosis) or increased endothelial permeability and neovascularization, thereby rendering meaningful clinical interpretation difficult. Conversely, the detection of specific biological processes involved in the initiation and progression of atherosclerosis such as inflammation, smooth muscle cell (SMC) migration, apoptosis and ECM remodeling could facilitate clinical data interpretation, enhance risk stratification and provide a means for therapy control. Specifically monitoring ECM changes by contrast enhanced magnetic resonance imaging (MRI) appears promising for the
assess the assessment of plaque burden in atherosclerosis or neointimal hyperplasia after angioplasty. Due to the lack of an established large animal model of coronary atherosclerosis and plaque rupture, we investigated a coronary injury model that we had developed and demonstrated previously with the use of MR lucent stents. Similar to native atherosclerosis, this model is characterized by inflammation, cellular proliferation, and remodeling phases.

Using this model, we have examined a novel elastin-specific, low-molecular weight contrast agent (BMS-753951, Lantheus Medical Imaging, Billerica, MA) for imaging vascular remodeling by magnetic resonance. Herein we demonstrate the feasibility of this approach in a swine model of endoluminal coronary injury through direct comparison of imaging observations to histomorphometric findings.

**Methods**

**Animal model and vascular injury**

Coronary stenting of the left anterior descending coronary artery (LAD) and balloon injury of the left circumflex artery (LCX) were performed in 6 female landrace pigs (30-35kg). The right coronary artery (RCA) served as the control vessel. Stent placement was performed with a mean balloon pressure of 10 atm and a mean stent diameter of 3.5 mm. To prevent local signal void artifacts as typically observed with currently used stainless steel stents, lasered MR-lucent prototype stents (Aachen Resonance, Germany) composed of copper (75%), silver (8%), platinum (2%), gold (14%) and palladium (1%) were used to allow artifact free imaging of the stent lumen and vessel wall. Stent implantation was followed by a 28-day normal diet. All experimental procedures performed on animals were approved by the German legislation on protection of animals. Pigs were fasted for 12 hours with free access to water and then sedated with ketamine (10-15 mg/kg BW), azaperone (2 mg/kg BW) and atropine (0.5-1 mg/k B) IM. Animals were then intubated after giving an intravenous bolus of propofol 1% (3-5mg/kg BW).
General anesthesia with controlled positive pressure ventilation was maintained with propofol 2% (8 mg/kg/h) and fentanyl given as bolus every 30 minutes. Vital parameters including arterial invasive blood pressure, oxygen saturation, capnography, body temperature and electrocardiogram were monitored throughout the entire experiment.

**Elastin-binding contrast agent**

BMS-753951 (Lantheus Medical Imaging, North Billerica, MA) is a low molecular weight MR contrast agent with moderate specificity for elastin (IC\(_{50}\) = 0.33 mM) and similar blood half-life time to currently used extracellular Gd-based MR–contrast-agents (<2%ID/g after 60 minutes). It is composed of a gadolinium-DTPA-chelate, which is linked to the d-amino acid D-phenylalanine. Ex-vivo and in-vivo pre-clinical data demonstrate preferential uptake in the arterial wall with rapid clearance from the blood pool by renal excretion. Its relaxivity increases upon binding to the arterial wall (4.5 mM-1 * sec-1 (unbound) vs. 13.7 mM-1 * sec-1 (bound to elastin)) thereby increasing the target to background signal ratio.

**Specificity of BMS753951 for elastin**

Different studies were pursued to demonstrate specificity of BMS753951 for elastin. To determine the gadolinium distribution of BMS753951 within the vessel wall, electron microscopy of elastic lamina was performed. BMS-753951 spectra at various locations of aortic vessel wall samples (N=3) were acquired to generate a spatially resolved BMS-753951 maps. Similarly, a carbon map was generated to localize the elastic fibres in the aortic samples, as elastin fibres are composed primarily of carbon. For this purpose the aortic samples were prepared by cryofixation against a liquid nitrogen cooled metal block. Cryosections were cut at -120ºC, transferred to Pioloform coated Ni-grids and freeze-dried over night. The sections were coated with a thin layer
of carbon, and viewed and analyzed in a FEI-Tecnai-12-electron-microscope equipped with an EDAXEDS detector. Mapping was achieved with the Edax-software (EDAX, UK).

Second, inductively coupled mass spectroscopy (ICP-MS) was performed on a subset of stented vessels (N=2) and control coronary segments (N=2) to quantify the amount of BMS753951 accumulation in the vessel wall. Vessel samples were digested in 70% nitric acid at 37°C overnight followed by dilution with deionized water for ICP-MS-analysis. A standard curve was run with each sample set for Gd-concentration determination. In addition, Elastica van Gieson (elastin) staining was performed in the stented, control and balloon injured coronary segments to visually assess elastin content.

At last, in vitro binding of BMS753951 to elastin was compared to other proteins as follows: \([^{153}\text{Gd}]\) BMS753951 was incubated with 5 mg/ml purified elastin from bovine cartilage or 5 mg/ml bovine serum albumin or 10 mg/ml of extracellular matrix protein chondroitin-6-sulfate. Free material was separated from bound by centrifugation (2000xg for 15 minutes) through a Centrifree® micropartition cartridge and measured by gamma counter (Wallac Wizard gamma counter, Gaithersburg, MD). The percent \([^{153}\text{Gd}]\) BMS753951 bound was plotted against the respective proteins\(^{22}\) (Figure 1).

**MRI protocol**

At day 28 after injury, free-breathing ECG-triggered coronary MR-angiography (MRA) \(^{24}\) and delayed gadolinium enhancement (LGE) imaging \(^{25}\) of the coronary vessel walls was performed using a 1.5T MR scanner (Achieva, Philips Medical Systems, NL) pre and post injection of 0.2mmol/kg Gd-DTPA (Magnevist, Bayer-Schering Health Care, Berlin). Animals were imaged in supine position using a 5-element cardiac phased array coil and an advanced cardiac software package (R2.1.3). After localization of the heart and determination of the mid to
end-diastolic rest period, a fast coronary scout scan was performed for identification of the course of the three major coronary arteries for subsequent high resolution coronary MRA and delayed enhancement coronary vessel wall imaging. Imaging parameters of the free-breathing-ECG triggered and navigator (NAV) gated inversion recovery (IR) segmented gradient echo vessel wall sequence targeted along the LAD, LCX and RCA included: FOV=320 mm, matrix=256x256, in-plane-resolution=1.25x1.25 mm, slice-thickness=3 mm, acquisition window=50ms, TR/TE=4.7ms/1.4ms, flip-angle=30º and slices=24. The inversion time (TI) for nulling blood signal was determined using a Look-Locker-sequence acquired in a coronal view of the heart. Data acquisition was synchronized with the mid to end-diastolic quiescent phase of coronary motion and navigator gating was performed using an end-expiratory 5mm gating window. Two days later, coronary MRA and delayed BMS-753951 enhancement (LBE) coronary-vessel-wall-imaging were repeated pre and 30, 60, 90 and 120 minutes post injection of 0.1 mmol/kg BMS-753951.

X-ray

After completion of MR imaging, X-ray coronary angiography was performed to assess the degree of luminal stenosis in the stented and balloon injured vessel segment. Subsequently, the animals were euthanized and hearts were harvested for histological analysis.

Image analysis

For objective image analysis, contrast-to-noise-ratio (CNR) between vessel wall and blood was determined by manual segmentation of the aortic and coronary vessel wall in the stented, balloon injured and control vessels on reformatted images. CNR was determined 30, 60, 90, and 120 minutes post-contrast-agent administration. For improved simultaneous visualization of focal contrast agent uptake and coronary anatomy, coronary MRA’s were manually registered.
and overlaid with the delayed enhancement images thereby providing fused images similar to image fusion performed in PET/CT.

Both the combined intima+media volume \( \text{volume}_{\text{intima+media}} = 2 \times \text{stent-length} / (3 \times (\text{area}_{\text{stent prox}} + 2 \times \text{area}_{\text{stent mid}} + \text{area}_{\text{stent dist}} + \sqrt{\text{area}_{\text{stent prox}} \times \text{area}_{\text{stent mid}}} + \sqrt{\text{area}_{\text{stent mid}} \times \text{area}_{\text{stent dist}}}) ) \) of the stented vessel wall and the combined intima+media area from histology were assessed. Areas of late enhancement detected by LBE-MRI were manually segmented in each slice and the volume was calculated by multiplication with the slice thickness. Combined media and intima volume were only assessed in the stented vessel wall, because only in those segments, significant enhancement allowed for reliable manual contouring.

**Histopathology and histomorphometry**

The coronary arteries were deep frozen or fixed in formalin immediately after explantation. Elastica-van-Gieson staining was performed in stented, control and balloon injured coronary segments to assess remodeling of the extracellular matrix. Elastin appears black on Elastica-van-Gieson staining.

The combined mean intima-media area was assessed in three slices, the proximal, mid and distal slice of the stented vessel segment and in one representative slice for the balloon injured and control vessel. As the severity of vessel injury by stenting strongly correlates with neointimal thickness and percent diameter stenosis \(^{26-29}\), the mean injury score for each section was measured as described by Schwartz et al.\(^ {27}\). 0=no injury; 1=break in the internal elastic lamina by stent strut; 2=perforation of the media by stent strut and 3=perforation of the external elastic lamina by stent strut.

**Statistics**
Results are expressed as mean±SD. A Student’s t test (unpaired, two-tailed) as applied for the comparison of continuous variables and a p-value<0.05 was considered statistically significant.

Results

Binding of BMS753951 to elastin

Previous studies indicate that $^{153}\text{Gd]}$BMS753951 binds to elastin.$^{22}$ This was examined further by electron microscopy, inductively-coupled–mass-spectroscopy (ICP-MS) and examining the binding of $^{153}\text{Gd]}$BMS753951 to purified elastin, serum albumin and of chondroitin-6-sulfate, another extracellular matrix protein.

Electron microscopy showed an excellent co-localization of targeted gadolinium $^{153}\text{Gd]}$BMS753951 with elastic fibers and the carbon map, as elastin fibres are composed primarily of carbon (Figure 2). With ICP-MS, a strong increase in Gd-concentration in stented coronary vessel segments (50-100μM) was observed compared to normal non-injured segments (10μM). In addition, in vitro binding studies showed that $^{153}\text{Gd]}$BMS753951 preferentially binds to elastin compared to the other two proteins (Figure 1).

These results are in good agreement with DE-MRI (Figure 3-6) and histological data (Figure 5, 6), showing increased elastin accumulation in the neointima of the stented vessel compared to the balloon-injured and control vessel segments.

MRI of coronary vessel wall and histological data

All animals were scanned without any adverse events. Strong aortic- and pulmonary artery-wall delayed enhancement (LBE) was seen after BMS-753951 administration (Figure 3, 5 and 6), which is in agreement with the high elastin content in those elastic vessels, while no visually apparent delayed enhancement (LGE) was observed after Gd DTPA injection (p<0.001).
In addition, after administration of BMS-753951, strong delayed enhancement (LBE) of the stented (Figure 3-5) and intermediate to little enhancement of the balloon injured (Figure 6) coronary vessel segment were observed. There was no to little visually apparent delayed enhancement in the control coronary artery apart from enhancement of the very proximal parts of both the RCA and left main coronary artery (Figure 6). No delayed enhancement of the stented, balloon-injured and control coronary vessel segments was observed on native inversion recovery images (Figure 3). After administration of Gd-DTPA delayed enhancement (LGE), coronary wall imaging showed no to little visually apparent enhancement in the injured vessel wall (stent or angioplasty) (Figure 3, 4) and no visually apparent enhancement in the control vessel.

Histological analysis revealed severe vascular remodeling in the stented segments (Figure 5, 7) with a mean injury score similar to other studies, where comparable arterial injury correlated with a considerable increase in neointima hyperplasia. The injury score showed also comparable stent expansion in all stented arteries (Table). In addition, only minor remodeling in the balloon injured segments in all animals was documented (Figure 6). This observation is consistent with the visual appearance of strong BMS-753951 delayed enhancement (LBE) in the stented and intermediate to little delayed enhancement (LBE) in the balloon injured coronary wall segments (Figure 3, 5 and 6). A good correlation was found between the combined media and intima volume by LBE-MRI and histology with a slight overestimation of 27% by MRI (Figure 7).

Quantitative analysis of vessel wall enhancement after administration of BMS-753951 yielded a 3-fold higher CNR (Figure 8) in the stented coronary artery when compared to the balloon-injured and control artery (p<0.05). No significant difference between the balloon-injured and control vessel was found. In addition, CNR between vessel wall and coronary blood was 3-fold increased in the stented coronary artery segment when compared to pre-contrast images approximately 30 minutes after BMS-753951 administration and remained at a
consistently high level in the stented coronary vessel wall segment suggesting strong association with the vessel wall with no wash-out tendency up to 2 hours post BMS-753951 injection (Figure 8). Planimetric evaluation of the combined media and intima area in the stented (proximal, mid, distal slice), balloon injured and control vessel (representative slices) correlated well with the contrast-to-noise ratio in those segments as measured by delayed enhancement MRI using BMS-753951 (Figure 8).

**X-ray coronary angiography**

Conventional X-ray single plane coronary angiography revealed no significant stenoses in the injured vessel segments in 5 animals. Only one animal showed a single 25-50% diameter in-stent-stenosis in the LAD.

**Discussion**

This study demonstrates that MRI with BMS-753951, an elastin-specific contrast agent, leads to signal enhancement in areas of severe vessel wall injury due to an increase in the combined media and intima area while no such findings were observed with conventional extracellular Gd-DTPA. The very low uptake of Gd-DTPA in normal and injured coronary vessel segments also suggests that the observed signal enhancement with BMS-753951 is unlikely related to a distribution volume effect as typically seen for Gd-DTPA but rather related to specific binding. This observation was also confirmed by electron microscopy, inductively coupled-mass-spectroscopy and the binding experiments to purified elastin. Delayed enhancement MRI with BMS-753951 showed significant higher signal enhancement in the stented coronary segment compared to the balloon injured and control artery, areas of enhancement were in good agreement with the increase in combined intima-media area as measured by histology. Histological analysis revealed considerable vascular remodeling in the
stented and only minor remodeling in the balloon-injured segments. Furthermore we observed strong uptake of BMS-753951 in the vessel wall of elastic arteries (no venous enhancement) with no washout tendency up to 2 hours after administration of the contrast agent. This novel approach may be useful for in vivo monitoring of vascular remodeling post mechanical injury. Additional studies with this approach are required to demonstrate the assessment of vessel wall thickness (plaque burden) in patients with suspected coronary artery disease (CAD). In addition, enhancement of elastic arteries such as the aorta and the pulmonary arteries was observed, whereas little uptake was found in native muscular arteries such as the control coronary artery segments.

ECM formation is the principal mechanism of restenosis in various experimental models and in humans after balloon angioplasty or stent placement and also contributes to the pathogenesis of atherosclerosis. Previous studies have shown that elastin is an important component of this process. Specifically, Brasselet has demonstrated that inhibition of elastin cross-linking resulted in a decrease in constrictive remodeling, and consequently restenosis, after arterial injury. Otherwise, elastin content is markedly increased following arterial injury. Moreover, in the study of Nili and co-workers, collagen and elastin were major contributors to ECM-synthesis after 21 and 60 days post injury, where the accumulation of elastin was attributed to an increased sensitivity to the mechanical forces of blood flow or balloon distension. Krettek and co-workers documented an ongoing but often ineffective elastogenesis in human atherosclerotic disease. Hence, the measurement of cumulative elastin content by MRI may be a promising technique for detection and quantification of ECM synthesis during arterial remodeling.

Noninvasive coronary-vessel-wall-imaging by MRI has already been demonstrated in pilot studies for in vivo risk stratification through quantification of subclinical coronary atherosclerotic plaque burden and outward arterial remodeling related to non-significant CAD.
However, a target-specific contrast agent for exclusive in vivo imaging of arterial remodeling would allow improved sensitivity and specificity for assessing processes directly associated with vessel wall repair or compensatory remodeling in atherosclerosis. In this regard, the present study demonstrated that non-invasive imaging of elastin formation by coronary MRI using an elastin-specific contrast agent is feasible and may be useful for non-invasive assessment of arterial remodeling after vascular injury. The finding of increased BMS-753951 uptake after coronary-vessel-wall-injury by LBE-MRI correlated well with the area of remodeling obtained from histological data. A good correlation between the volume of remodeling by histology and the volume of delayed enhancement was found in the stented vessel wall with MRI slightly overestimating (27%) the intima-media volume. This overestimation most likely results from the limited spatial resolution of the data sets as shown by Schar et al.\textsuperscript{33}

Taken together, contrast enhanced coronary-vessel-wall-imaging by MR may be a promising technique for diagnosis of early inward or outward remodeling post mechanical injury. Further studies are required to evaluate the pertinence of these findings in patients with multiple cardiac risk factors and a high likelihood of subclinical or advanced atherosclerosis.

**Comparison with other imaging techniques**

While x-ray-angiography and multi-detector-CT (MDCT) provide excellent spatial resolution and morphologic details in a fraction of time required for coronary MRI, they suffer from low sensitivity for contrast agent detection and therefore are limited in their ability to detect biological alterations in the vascular wall. Nevertheless, MDCT has been shown to provide valuable information about certain plaque components (soft, fibrous, calcified plaque)\textsuperscript{34} and information on vessel wall remodeling in patients with documented CAD\textsuperscript{35}. It remains to be seen whether MDCT, especially with the advent of dual energy MDCT or multi spectral CT, will play a major role in the biological characterization of the vascular wall in the future.
Limitations

Coronary in-stent-stenosis cannot be directly assessed by MRI because of the local signal void caused by susceptibility induced background gradients or RF shielding of currently used stainless steel stents. In our experimental setting we used a MR-lucent prototype stent to allow artifact free imaging of the stent lumen and vessel wall. Hence, at present, this technique is not feasible in a clinical setting.

As our model did not produce significant in-stent-stenosis as demonstrated by x-ray, signal enhancement was mainly related to positive remodeling of the stented area. The sensitivity for minor remodeling is limited by spatial resolution and partial volume effects. For those reasons and as can be seen in Figure 7, we only quantified the area of remodeling by CE-MRI for the stented segments, in which significant signal enhancement could be observed.

In addition, we used an animal model without underlying atherosclerosis. Thus, results obtained in this study may differ from patients with advanced CAD. Furthermore, elastin is present in both the pathologically altered and normal vessel walls of highly elastic vessels such as the aorta and the pulmonary arteries and thus further studies are required to better understand the clinical meaning of the observed increase in elastin signal in patients with sub-clinical and advanced CAD with and without intervention.

The circumflex coronary artery is sometimes more difficult to image with MRI due to its posterior location and the resulting lower CNR. This may have caused a slightly lower CNR in the balloon-injured vessel when compared to the stented LAD but is unlikely to explain a 3-fold lower CNR as measured in this study.

As this novel contrast agent binds to both normal as well as immature de novo (tropo)elastin (synthesized as a response to vessel injury), the observed signal enhancement after BMS-753951 administration depends on the natural variability of mature elastin fibers in the
coronary arteries. In this study we observed a significantly and consistently higher CNR in the stented vessel segment (CNR~20) when compared to the control and balloon-injured vessel segments (CNR~5-7) suggesting de-novo (tropo)elastin formation in the stented vessel segments as also demonstrated by histology. However, distinguishing newly formed (tropo)elastin from mature elastin is not feasible with this method. Finally, the contrast agent used in this study is not approved for clinical application at the present time.

Conclusions

We demonstrate the feasibility of non-invasive assessment of vascular remodeling in an animal model of coronary vessel wall injury using a novel, low-molecular weight elastin-binding gadolinium chelate and MR-lucent stents. The presented technique may have applications for the detection of pathologic alterations of the ECM in patients with sub-clinical and advanced CAD. Further studies systematically relating elastin content MR signal to enhancement following BMS-753951 administration in non-atherosclerotic and atherosclerotic animal models and degenerative ECM disease are now warranted.
Acknowledgments

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Disclosures

There are no ethical problems and no conflicts of interest for any of the authors related to the material. The authors Simon Robinson, Joel Lazewatsky, Richard Cesati and David Onthank are employees of Bristol-Myers-Squibb (North Billerica, MA, SA). The author Arno Buecker is Cofounder of Aachen Resonance.
References


Table. Morphometric variables

<table>
<thead>
<tr>
<th>Animal-No.</th>
<th>Stented artery</th>
<th>Injury score*</th>
<th>Intima/Media vol (mm³)</th>
<th>Neointima area (mm²)</th>
<th>Intima/Media area (mm²)</th>
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<tr>
<td>1</td>
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<tr>
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<td>2,65</td>
<td>4,30</td>
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<tr>
<td>5</td>
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<tr>
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<td>207,1</td>
<td>2,60</td>
<td>4,25</td>
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</tbody>
</table>

Table shows the morphometric variables with the mean injury score and the mean neointima area in stented arteries. Histomorphometric analysis of animal 5 revealed a chronic total coronary artery occlusion (CTO), which did not allow defining an injury score. * Injury score was defined as followed: 0=no injury; 1=break in the internal elastic lamina by stent strut; 2=perforation of the media by stent strut and 3=perforation of the external elastic lamina by stent strut.
Figure Legends

**Figure 1.** Binding of [153Gd]BMS753951 to purified elastin, bovine serum albumin (BSA) and chondroitin-6-sulfate. [153Gd]BMS753951 preferentially binds to elastin compared to BSA and chondroitin-6-sulfate, another extracellular matrix protein.

**Figure 2.** BMS-753951 spectra at various locations of an aortic vessel wall sample were acquired to generate a spatially resolved BMS-753951 map (C). Similarly, a carbon (B) map was generated to localize the elastic fibres in the aortic sample. Elastin fibres are composed primarily of carbon. A transmission electron microscopy image (A) of the aorta shows the course of one elastic fibre (white arrow, black box). Good co-localization between BMS-753951 distribution (C), carbon and elastic fibres can be observed (white arrows).

**Figure 3.** Coronary MRA (A, E) of stented LAD pre (B, F) and post injection of Gd-DTPA (C, G) and after administration of BMS-753951 (D, H). No or minor delayed enhancement is visible after admission of Gd-DTPA, whereas strong enhancement is present in the stented area of the LAD after administration of BMS-753951 (D, H). Strong enhancement of aortic and pulmonary artery (D) after BMS-753951 consistent with the high elastin content of those vessels. Non-specific accumulation of Gd-DTPA (C, G) and BMS-753951 (D, H) can be seen in the cartilage of the ribs. White arrows with hidden line point out the stented area with amplification in the right lower corner (D, G, H).

**Figure 4.** Contrast–to-noise-ratio (CNR) >30 minutes after application of GD-DTPA and BMS753915 in the stented coronary artery segment.
**Figure 5.** Comparison of coronary MRA (A), delayed enhancement MRI (B) and PET-CT-like fusion of A and B (C) of stented and control coronary vessel segment and corresponding histology (D-G). Strong enhancement can be observed at the stent location (dotted white arrow) while no to little enhancement is visible in the normal non-injured LAD segment (B, C). Elastic-von-Gieson (EvG) stain of non-injured coronary vessel segment (D, E) shows intact internal elastic lamina (IEL) and circular arranged elastin fibers (black) in the media. EvG of stented vessel segment (F, G) demonstrates disruption of IEL and neointima formation with diffuse elastin deposition (black dots). (E, G) Magnifications of (D) and (F). MRA: magnetic resonance angiography, MRI: magnetic resonance imaging, LAD: left anterior descending, A: adventia, M: media, L: lumen and N: neointima.

**Figure 6.** Coronary MRA of control vessel (RCA) and balloon-injured LCX demonstrating lumen integrity at the site of injury (A). Comparison of contrast enhancement between balloon-injured LCX and non-injured RCA demonstrating intermediate to little enhancement of the balloon-injured LCX and no to little enhancement of the control vessel segment (B). Corresponding histological sections stained with EvG demonstrate intact internal elastic lamina (IEL) in control vessel and circular elastic fibers in the media. In contrast, disruption of IEL was observed at the site of balloon injury accompanied with diffuse deposition of elastin in the neointima (D). MRA: magnetic resonance angiography, LCX: left circumflex, RCA: right coronary artery, A: adventia, M: media, L: lumen and N: neointima.

**Figure 7.** Fusion of coronary MRA (LAD) and delayed enhancement images (B) demonstrates strong uptake in the stented segment. Red lines indicate location of histological sections relative to stented vessel area. Scatter plot (A) showing correlation between volume of
enhancement after administration of BMS-753951 and volume of combined intima+media by histology. Photomicrographs of histological sections from the distal (C), mid (D) and proximal (E) part of the stented vessel wall (Elastica-van-Gieson stain). L: lumen. M: media. N: neointima. S: strut.

**Figure 8.** Contrast-to-noise ratio (CNR) in the stented, balloon-injured and control coronary artery vessel wall pre and post-injection of BMS-753951 (A). CNR in the stented coronary vessel wall increased already 30 minutes after BMS-753951 injection and even slightly increased over a 2-hour period suggestive for specific binding to elastin in the injured vessel wall. (*) p-value<0.05, (†)p-value<0.01. Scatter blot (B) showing correlation between vessel wall contrast-to-noise (CNR) and combined intima-media area. L: lumen. Triangle=control, square=balloon injured vessel, trapezoid=stented vessel.
p<0.001

CNR > 30 minutes

stent Gd-DTPA  stent BMS753951

American Heart Association
MRI of Coronary Wall Remodeling in a Swine Model of Coronary Injury using an Elastin-Binding Contrast Agent

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