Targeting of Matrix Metalloproteinase-2 activation with Gd-NBCB-TTDA-MMP-2 for detection of vulnerable atherosclerotic plaques using a novel molecular MR Imaging in vivo

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Abstract

We successfully synthesized and characterized a novel gadolinium-based MR contrast agent, Gd-NBCB-TTDA-MMP-2 , that molecularly targets MMP-2.The aim of this study was to image MMP-2 activity in vivo in atherosclerotic plaques of ApoE -/- mice with Gd-NBCB-TTDA-MMP-2 enhanced MRI. Gd-NBCB-TTDA-MMP-2(D), a scrambled form of Gd-NBCB-TTDA-MMP-2, enhanced MRI was also performed as a comparison. Our results showed that Gd-NBCB-TTDA-MMP-2 targeted to MMP-2 facilitated in vivo specific assessment of atherosclerosis and its plaque vulnerability.

Introduction

Characterization of plaque composition represents a new goal for noninvasive identification of vulnerable lesions and many cellular/molecular imaging agents are currently being evaluated. An increased expression and activation of MMP-2 has been revealed in atherosclerotic plaques (1), most prominently in vulnerable regions (2, 3), suggesting a pathogenic role of MMP-2 in the progression of plaque rupture. (4) Vulnerable plaques may show characteristic morphologic features, however, there are differences in their biology and activity, which ultimately leads to rupture. As a consequence, considerable efforts have been undertaken to identify biologic mechanisms of atherosclerotic lesions by use of molecular-targeted probes. Nevertheless, molecular imaging of atherosclerosis is still a work in progress. The goals of this study were to assess and validate that Gd-NBCB-TTDA-MMP-2 targeted to MMP-2 facilitated in vivo assessment of atherosclerosis at a molecular pathologic level and to evaluate the specificity of Gd-NBCB-TTDA-MMP-2 for MMP-2 by comparing to that of Gd-NBCB-TTDA-MMP-2(D) (untargeted, scrambled form) by using a 3.0 T clinical MR scanner (Signa; GE) with a high-resolution animal coil.

Methods

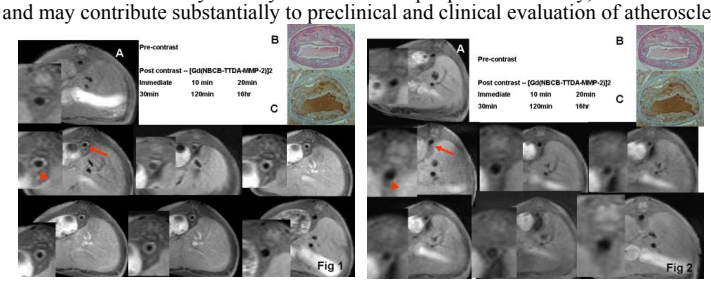
The derivative of TTDA, NBCB-TTDA, introducing p-nitrobenzyl and cyclobutyl groups was synthesized. Gd-NBCB-TTDA-MMP-2 was synthesized by conjugated a gadolinium chelate to MMP2 peptide. Gd-NBCB-TTDA-MMP-2(D) (control contrast agent) was also synthesized by replacing the targeting moiety peptide of L-Valine by D form. These two Gd(III) complexes were characterized by relaxivity r_1 and in vitro MR image. Gd-NBCB-TTDA-MMP-2 and Gd-NBCB-TTDA-MMP-2(D) were injected into atherosclerotic ApoE -/- and wild-type mice. The enhancement of atherosclerotic plaques in aorta produced by the contrast agents was measured on MR images at immediate, 10, 20, 30 minutes and 16 hours after injection. The interval of the two contrast examinations were more than ten days. Post contrast sections were precisely anatomically matched with the sections obtained with the preinjection baseline image. The MR images were analyzed with contrast-to-noise (CNR), the normalized enhancement ratio (NER) and percentage NER (5).Statistical Analysis was performed with paired t test and one-way analysis of variance. MMP-2 expression was investigated in the aortas by using MMP-2 immunostaining and in situ MMP zymography.

Results and Discussion

^{1}H NMR spectrum, mass spectrum and HPLC separation data showed correct synthesis of the Gd-NBCB-TTDA-MMP-2.The relaxivity r_1 time of the Gd-NBCB-TTDA-MMP-2 was 5.29 mM^1s^1. The in vitro MR images showed increased signal of Gd-NBCB-TTDA-MMP-2 cleaved by MMP-2 enzyme (complex+ HSA+ enzyme).In Vivo MRI imaging of MMP-2 expression showed significantly enhancement of the aortic wall in the high-cholesterol-fed ApoE -/- mice after Gd-NBCB-TTDA-MMP-2 administration (Fig. 1) and mild enhanced after Gd-NBCB-TTDA-MMP-2(D) administration (Fig. 2). No significant enhancement was observed in the control wild-type mice. In vivo MR image signal intensity (SI) of aortic wall at different time points measured with percentage NER (Figure 3) and CNR (Figure 4) showed that MMP2-targeted Gd-NBCB-TTDA-MMP-2 facilitated a 52.1% increase (P< .001) in percentage NER of atherosclerotic lesions (CNR, 34.4 compared with 19.2; P< .001). Nontargeted Gd-NBCB-TTDA-MMP-2(D) provided 26.97% increase in percentage NER (CNR, 21.8 compared with 21.6; P> .005). Aortic sections immunostained for MMP-2 showed high level staining (Fig 1C, 2C) that indicates a substantial level of MMP-2 in atherosclerotic lesions of ApoE -/- mice. We believe that this molecular imaging technique will explore the feasibility and validation of in vivo MR imaging of inflammation in atherosclerotic lesions by visualization of MMP-2 activity in mice.

Conclusion

Our study demonstrated that Gd-NBCB-TTDA-MMP-2 targeted to MMP-2 facilitated in vivo specific assessment of atherosclerosis at a molecular pathologic level. Because MMP2 activity directly correlates with plaque vulnerability, noninvasive assessment of MMP2 activity is expected to allow identification of unstable plaques and may contribute substantially to preclinical and clinical evaluation of atherosclerosis.



In vivo MR images acquired from the same high-cholesterol-fed ApoE -/- mice. (Red arrow head shows enlargement of aorta)
(Fig1): Prominent enhancement in the atherosclerotic plaques with Gd-NBCB-TTDA-MMP-2 at different time points
(Fig.2) Mild enhancement in the atherosclerotic plaques with Gd-NBCB-TTDA-MMP-2(D) at different time points
(B) Hematoxylin-eosin stained (C) MMP-2 Immunohistochemical stain

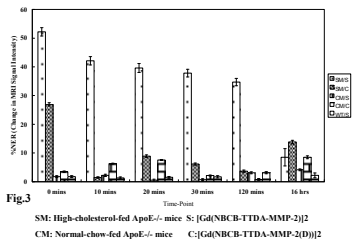


Fig.3

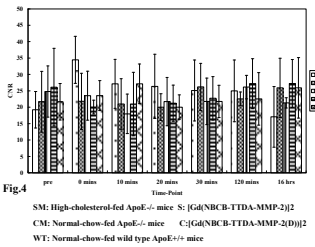


Fig.4

Graphs depict quantitative temporal MR imaging results. In vivo MR image SI of aortic wall at different time points measured with (Fig. 3) percentage NER and (Fig.4) CNR

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