Novel Human Myeloperoxidase-sensitive Paramagnetic Substrate for MR Signal Amplification

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Introduction

Recent evidence shows that atherosclerosis is an inflammatory response to injury [1, 2]. Unlike more benign type lesions, in high risk plaques, activated macrophages produce myeloperoxidase (MPO) in response to vascular injury. Myeloperoxidase consumes hydrogen peroxide and generates hypochlorite, a pro-oxidant that contributes to erosion and rupture of plaques. We hypothesized that by using paramagnetic electron donor compounds that rapidly oxidize and polymerize in the presence of MPO one could detect the presence of the enzyme in tissues by magnetic resonance imaging.

Materials and methods

The myeloperoxidase sensitive probe was synthesized by covalently linking 3-(2-aminoethyl)-5-hydroxyindole (HIEA, serotonin oxalate, Fluka) with DOTA(Gd)methylglycine provided by Schering AG, and purified using C-18 reverse-phase HPLC. Human neutrophil MPO was obtained from Biodesign. Glucose oxidase and hydrogen peroxide were obtained from Sigma. The kinetics of the conjugation was measured on a Hitachi 3000 UV/vis spectrophotometer. The longitudinal relaxation rates of aqueous samples of the substrate with and without the presence of hydrogen peroxide and human MPO were determined at 0.47 T on a Bruker Minispec NMR spectrometer and at 1.5 T on a GE whole body MRI scanner by the inversion recovery technique. Matrigel™ samples were prepared by first incubating 250 µL of Matrigel™ with various amounts of glucose oxidase and MPO on ice and poured into the wells of a 96-well plate. The gels were formed by incubating the plate at 37°C. Substrate solutions (HIEA-DOTAGd, 650 nmol/ml) were poured on top of the Matrigel™. Control samples without glucose oxidase, MPO, or both were also prepared. Imaging experiments of the Matrigel™ samples were performed on a GE 1.5 T whole body MRI scanner using spin echo and inversion recovery sequences at 1 hour and 24 hours after sample preparation. T1 and T2 relaxation times were determined using a nonlinear least squares fitting program.

Results and disucssion

We have previously reported a substrate that polymerized in the presence of horse radish peroxidase, tyramide-GdDOTA [3]. However, this substrate reacted slowly with MPO, and thus represents a weak substrate for MPO. We have designed and synthesized a novel MPO-responsive "smart" probe consisting of a covalent conjugate of GdDOTA analog with (3-(2-aminoethyl)-5-hydroxyindole). The obtained probe (HIEA-GdDOTA) was shown through T1 measurements and spectroscopy to rapidly oxidize in the presence of human neutrophil MPO and hydrogen peroxide. The oxidized product is an oligomer that demonstrated a 2 fold increase of R1 (gadolinium longitudinal relaxivity) at 0.47 T and 1.5 T in aqueous solutions.

Utilizing a Matrigel™ tissue model system and using glucose/glucose oxidase as the hydrogen peroxide generating system (peroxide is released by neutrophils *in vivo*), we observed an MPO-specific change in the MR signal (Figure 1) that is visible at 1 hour after sample preparation. The changes continued to increase over time, and at 24 hours, there is a 30% increase in the longitudinal relaxation rate (5.3 s^{-1} to 6.9 s^{-1}) and a 20% increase in the transverse relaxation rate (8.8 s^{-1} to 10.6 s^{-1}). Only the interface between the gel and the substrate solution demonstrated signal change. No such change was identified in control systems where either MPO, hydrogen peroxide, or both were not present (first cell, Figure 1) These findings are consistent with the accumulation of MPO-converted oligomers at the interface, since because of the larger molecular sizes, the MPO-converted products cannot diffuse rapidly in the Matrigel™. This results in the delineation of the interface between the gel and the unconverted substrate solution. Interestingly, there was visible signal change at the interface not only on T1 weighted (not shown) but also on T2 weighted spin echo and inversion recovery images (Figure 1), and the changes are slightly more prominent on inversion recovery images. As T1-, T2-weighted and inversion recovery (e.g., STIR, FLAIR) sequences are widely used clinically, T1, T2, and IR MPO-mediated contrast-enhanced images may be used to improve diagnostic sensitivity in detecting advanced atherosclerosis.

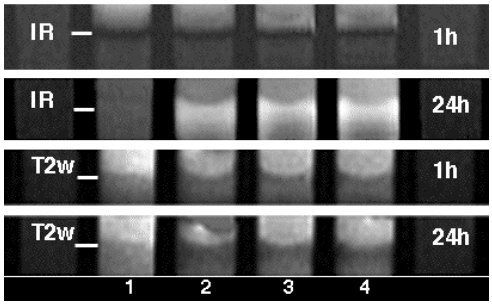

Figure 1: 1- control, no enzyme. 2-4 contained 6 µg MPO and 650 nmol HT-DOTAGd/ml and 2- 0.1 µg GO, 3- 0.5 µg GO, 4- 1 µg GO. IR: TR=700ms, TI=200ms, TE min. T2W: TR=300ms, TE=200ms.

Conclusions

We have synthesized, characterized, and validated the first myeloperoxidase sensitive MR imaging agent in a realistic tissue phantom study.

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References

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