In vivo Overhauser-enhanced MRI of Proteolytic Activity

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Purpose: Molecular imaging is of increasing interest in the field of pathology diagnosis, prognosis and monitoring. Among the existing imaging techniques available nowadays, MRI is a promising method for future molecular imaging applications due to its noninvasive nature, high spatial resolution and remarkable soft tissue contrast. However its sensitivity is quite low. To counteract this weakness, a new approach, Overhauser-enhanced MRI (OMRI) was developed at 0.2T[1]. This technique is based upon the Overhauser effect occurring while unpaired electrons of a free radical species interact with surrounding water protons (^{1}H) after saturating electron spins. Nuclear ^{1}H polarization is then increased, thereby, revealing the presence of unpaired electrons in MR images. Here, our aim was to visualize a proteolytic activity happening inside living mice during digestion. In this perspective, a nitroxide-labeled macromolecule that can generate strong Overhauser enhancements upon cleavage was designed (Figure 1). This current work would eventually be transposed into disease-bearing animal model with a view to early diagnosis.

Methods: OMRI Setup: It was composed of a 0.2T MRI system (Magnetom Open Viva Siemens) with a C-shaped resistive magnet (8.25MHz ^{1}H frequency) and a Transverse-Electric TE011-mode resonant cavity (5.43GHz electron frequency, Bruker) placed at the centre of the magnet.
Animal Preparation: 200µl of prepared nitroxide-labeled elastin at 18mM was orally administered in mice (n=7, CB57/CRL). They were then anesthetized and placed on a thermostatic bed for imaging.
Imaging Protocol: **1)** A 17-minute gradient echo anatomical image – FLASH3D (resolution = 0.5×0.5×0.5 mm^3) was obtained. **2)** Successive 18-second images were acquired using a fully balanced steady-state sequence - TrueFISP3D (resolution = 0.5×1×1 mm^3) **with/out** electron spin saturation. A keyhole acquisition paradigm was used. **3)** A 5-minute TrueFISP3D image of resolution 0.5×0.5×0.5 mm^3 without electron spin excitation was obtained for keyhole reconstruction.
Image Processing: 3D keyhole reconstruction resulted in OMRI images with electron spin saturation (OMRI-on) or without (OMRI-off). Parametric images of Overhauser enhancements were then calculated as a ratio of signal intensity of OMRI-**on** images to that of OMRI-**off**.

Results: Overhauser enhancements of 7.2 ± 2.4 (n=7) were observed at the opening of the duodenum about 20-30 minutes post-gavage where the pancreatic juice is first secreted by the exocrine pancreatic cells (Figure 2). No Overhauser enhancements were observed in the stomach (Figure 2). The 3D superimposed images were obtained from the anatomical image and the reconstructed 3D keyhole OMRI-**ON** image. This overlay validated the correct localization of the Overhauser enhanced signal within the digestive tract of the mouse.

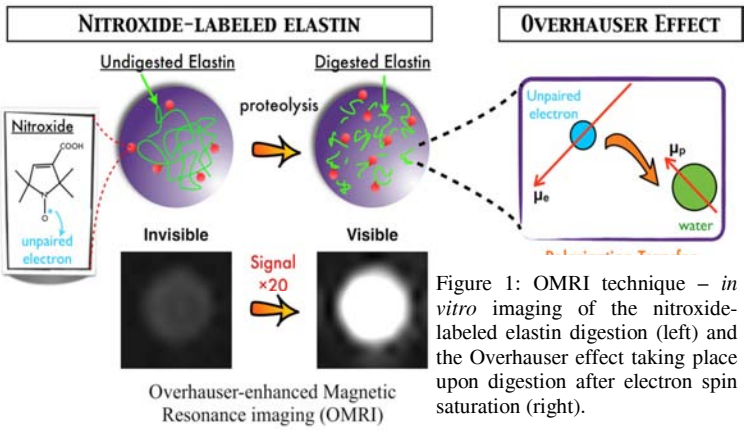

Figure 1: OMRI technique – *in vitro* imaging of the nitroxide-labeled elastin digestion (left) and the Overhauser effect taking place upon digestion after electron spin saturation (right).

Discussion: In this study, highly resolved OMRI of the mouse's abdomen, with spatial resolution 0.5×0.5×0.5 mm^3 in less than 20s acquisition was achieved (5 times quicker than the previous article[2]). Specific pancreatic elastin proteolysis was observed in the duodenum with strong signal amplifications. In conclusion, OMRI can be used to target abnormal proteolysis using specific radical probes.

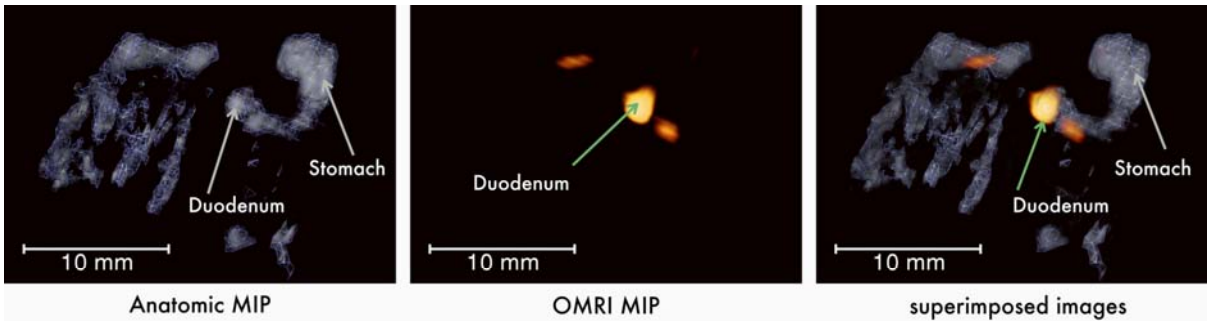

Figure 2: *In vivo* proteolysis 26 minutes post-gavage. Color maps of Maximal-intensity-projections (MIP) of anatomical 3D-FLASH image (left), the reconstructed 3D keyhole OMRI-on image (middle) and superimposed image (right).

References: 1. Mellet.P et al. New concepts in molecular imaging: non-invasive MRI spotting of proteolysis using an Overhauser effect switch. PLoS One 2009;4(4):e5244. 2. Massot.P et al. *In vivo* high-resolution 3D overhauser-enhanced MRI in mice at 0.2T. Contrast Media Mol Imaging 2012 Jan-Feb;7(1):45-50.