**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. This technique is based upon the Overhauser effect occurring while unpaired electrons of a free radical species interact with surrounding water protons (¹H) after saturating electron spins. Nuclear ¹H 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 ¹H 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 mm³) was obtained. 2) Successive 18-second images were acquired using a fully balanced steady-state sequence - TrueFISP3D (resolution = 0.5×1×1 mm³) 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 mm³ 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.

**Discussion:** In this study, highly resolved OMRI of the mouse’s abdomen, with spatial resolution 0.5×0.5×0.5 mm³ in less than 20s acquisition was achieved (5 times quicker than the previous article²). 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.

**References:**