

## Macromolecular Dynamic Contrast Enhanced (DCE) MRI characterizes hyperpermeability of the intestinal microvasculature in a Colitis model

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### Introduction

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the colon and small intestine that, among other things, results in alteration and dysfunction of the intestinal microvasculature. The goal of this work was to image and characterize blood vessels in the colon in an animal colitis model and to develop a protocol for detection of alterations of the microvasculature in colitis.

### Methods

C57 black mice were exposed to DSS (dextran sulfate sodium, an irritant to the colon; n=5) in the drinking water for 7 days. Controls (n=5) were given regular drinking water. After 7 days the mice were imaged at 9.4T BioSpec, using a quadrature resonator for excitation and detection (Bruker, Germany). Macromolecular Biotin-BSA-GdDTPA, as contrast agent, was prepared as reported previously (Dafni et al., 2002) and injected through a tail vein catheter as bolus. 3D gradient echo (3D-GE) images of the lower abdomen were acquired before and sequentially for 30 minutes after iv injection of the contrast agent. A series of variable flip angle precontrast T1-weighted 3D-GE images were acquired to determine the endogenous precontrast R1. The mice were imaged for 30 minutes after contrast injection and subsequently, a few minutes before euthanasia, iv injected with of BSA-ROX, as an early vascular marker. Pixel-by-pixel analysis was done on a PC using MATLAB software to generate the concentration of biotin-BSA-GdDTPA in a ROI that contain colon for selected slices. 3D rendering was done a Mac with OsiriX software. The histological sections of the colon were stained with fluorescein-labeled avidin (avidin-FITC) for visualization of the biotinilated contrast agent and with H&E for gradation of the disease.

### Results

In the colon of the control mice, contrast agent shows slow clearance, since blood vessels in the healthy colon are not leaky. The colon of mice with colitis showed exponential rate of enhancement due to accumulation of macromolecular contrast agent. The data are consistent with substantial extravasation of plasma proteins (such as the BSA-based contrast media) from colon vasculature (Fig. 1). Post contrast, selective enhancement of the colon was observed in the mice with colitis compared to control animals (Fig. 2). On H&E stainings, all animals with colitis were scored with severe multifocal necro-ulcerative colitis affecting approximately 70% of the available sample. The staining of the contrast agent, with avidin-FITC, confirmed substantial extravasation of the macromolecular contrast agent, containing albumin, whereas the early vascular marker BSA-ROX highlighted the blood vessels only.

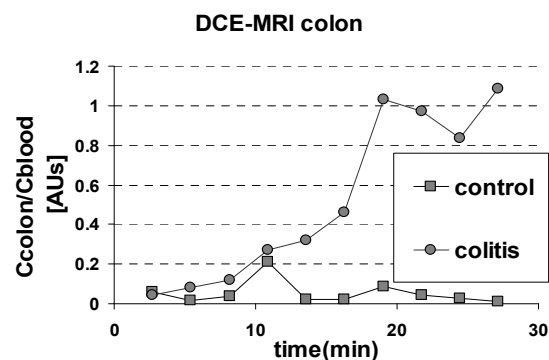


Fig. 1. Dynamic 3D imaging included follow-up of contrast enhancement for 30 minutes post contrast within the colon. Concentration of contrast material in the selected ROI (colon) was normalized by the concentration in the cephalic vein.

### Discussion

Here, we showed with non-invasive macromolecular DCE-MRI, plasma protein leakage to the colon, highlighting the focal patches of colitis in post contrast 3D rendering (Fig. 2). Macromolecular DCE-MRI demonstrated to be able to identify severe colitis and the loss of plasma proteins. Fluorescence microscopy validated the vascular leakage of the colon microcirculation into the lumen. Although not macromolecular, leakage of low-molecular GdDTPA into the lumen of the colon has been shown before, due to enhance vascular leakiness and explained as increased luminal fluid resulting from inflammatory disease (Alexopoulou et al., 2009; Young et al., 2009). Leakage of plasma proteins and deposition of a provisional matrix can support inflammation and stimulate remodeling of the colon vasculature.

**Acknowledgment** EU-FP6 MEDITRANS

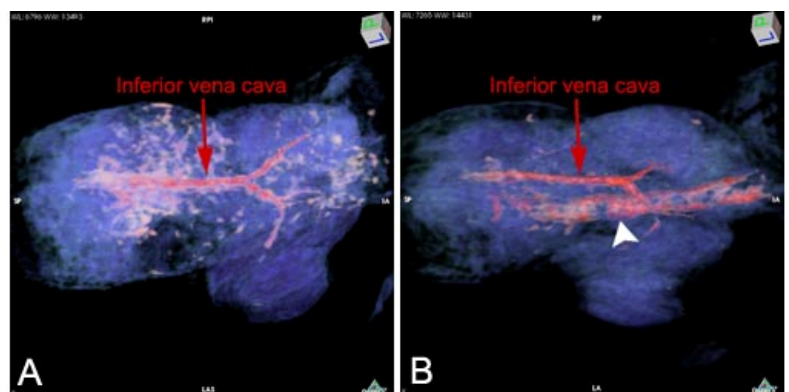


Fig. 2. 3D rendering of the lower abdomen of a healthy mouse (A) and a mouse with colitis (B) 30 min after iv injection of macromolecular contrast agent. The white arrow in B shows selective enhancement of the colon due to extravasation of biotin-BSA-GdDTPA.