Phase Contrast MR Imaging to Image Bacterial translocation in a Mouse Model for Graft versus Host Disease

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Target Audience

Clinicians that are interested in novel tools for the tracking of bacterial infection in vivo. MRI scientists that are interested in imaging using SPIO-mediated contrast with high susceptibility weighted contrast (SWI).

Purpose

Tracking of labelled bacteria in vivo could help to understand several disease mechanisms e.g. Graft versus host disease (GvHD). Since neutrophils are recruited rapidly to sites of bacterial infection, we considered the possibility that in the pathogenesis of GvHD neutrophils accumulate as a result of bacterial leakage into the intestine and the surrounding tissue. In this study we wanted to show that it is possible to track bacterial infection directly in vivo after total body irradiation (TBI) using SPIO-tagging of bacteria and highly sensitive Phase contrast MRI [1].

Methods

E. coli labelling with SPIO: We have established a procedure to label gramnegative E.coli bacteria, with superparamagnetic iron oxide particles (SPIO) particles (Ferucarbotran, nanoPET-Pharma, Berlin, Germany) consisting of SPIO nanoparticles (average diameter, 65 nm, and core size, 3-5 nm) coated with carboxydextran. We exposed E. coli to a SPIO concentration of 2µl/ml for 24h and performed 2 consecutive washing steps. The general feasibility of gramnegative bacterial labeling and tracking with MRI has already been shown [2].

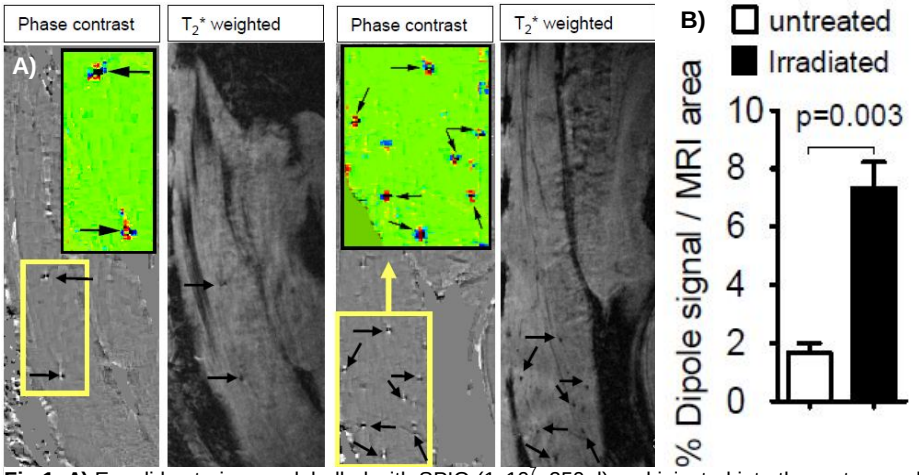

Fig.1: **A)** E. coli bacteria were labelled with SPIO ($1x10^7$, 250µl) and injected into the rectum and lower colon and traced by MRI in untreated animals and 24h after TBI, respectively. MRI detects $SPIO^+$ E. coli in the phase contrast (left) and the T2* weighted imaging (right) of the periintestinal tissue with the typical dipole shape pattern more prominent on the high-pass filtered phase images (arrows, magnified view). **B)** Quantification of dipole signals in untreated mice or mice having undergone TBI is displayed. The experiment was performed twice and the data are pooled, n=6 per group. Data are presented as mean and SEM.

MRI procedure: MRI experiments were performed on a Bruker 7 T Biospec system using a cryogenically-cooled quadrature-resonator (Bruker, Ettlingen, Germany). $SPIO^+$E. coli were injected into the rectum of mice that had received TBI or no treatment. MRI of mice was performed at serial time points (0h, 4h, 24h) The protocol consisted of a 3D FLASH sequence (TR/TE = 20/3.04 ms, resolution 50 × 52 × 52 µm^3, FA = 10°, bandwidth 55 kHz, acquisition time 9 min 12 s) and a 3D gradient echo (GE) flow compensated sequence (TR/TE = 120/10 ms, FA = 22°, spatial resolution 40 × 40 × 250 µm^3, bandwidth 28 kHz, acquisition time 8 min 57 s). Both sequences were chosen to provide morphological information about the migration of $SPIO^+$ E. coli, based on their R_2 relaxation rates and their magnetic susceptibility, respectively.

Data post processing: GE flow compensated images were processed offline with custom-made software developed in Matlab to reconstruct both magnitude and phase images. Local phase changes are visible due to susceptibility difference between the bacteria labeled with SPIO particles and the surrounding tissue. This difference produces a local discontinuity in the magnetic field with high spatial variations over a small distance at the interface between the SPIO particles and the neighboring intestinal tissue. Consequently, a high pass filter of the phase image will allow suppression of all slowly varying effects and depict only the effects originating from the susceptibility differences. The high-pass filtering operation consists of two steps: (1) a low pass filter of the complex image using a gaussian filter (equal to the image size) with a normalized σ = 0.14 and (2) complex division of the complex image by the low pass filtered image on a voxel by voxel basis.

Results

After 24 h the black signal derived from $SPIO^+$E. coli was gone from the rectum but signals from $SPIO^+$ E. coli creating a dipole-shaped pattern was clearly visible in the tissue surrounding the intestinal wall. $SPIO^+$E. coli were seen significantly more frequently in mice that had received TBI as compared to the untreated group indicating that TBI enhanced transmigration of bacteria through the epithelial layer of the intestinal wall (Fig. 1). Histology confirmed the presence of SPIO-particles in periintestinal and lymphatic tissue (Fig.2).

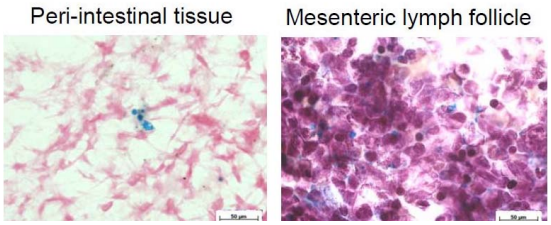

Fig. 2 Prussian blue staining indicates SPIO iron particles in the periintestinal area and in mesenteric lymph follicles in mice undergoing TBI.

Discussion/Conclusions

In this study we demonstrate that it is possible to track the translocation of E. coli bacteria labelled with SPIOs from the rectum into periintestinal tissue using MR phase imaging in mice that had received TBI. The resulting phase image (Fig. 1A) preserves the small-scale phase variations caused by local superparamagnetic iron oxide particles yielding a typical dipole-shaped pattern. Compared to the conventional T_2^* magnitude images, this technique ensures better monitoring of bacteria migration and leakage from the intestines and of their tissue biodistribution.

References

[1] Haacke et al. Magn Reson Med, 2004

[2] Wang et al. Nature Preceedings, 2009