T2- and Perfusion-Based MRI Assessment of Colon Wall and Mesenteric Inflammation in the Rat

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Introduction: Rectal installation of TNBS (trinitrobenzenesulfonic acid, an immunological hapten) and ethanol (a mucosal barrier breaker) in rodents is an established model for human inflammatory bowel disease (IBD), producing colonic inflammation and marked thickening of the bowel wall persisting for at least 1-2 weeks. We have developed MRI and image analysis methods to study the inflammatory changes in Wistar rats.

Materials and Methods: Animal experiments complied fully with UK ethical and legal requirements. ***Protocol.*** Rats were fasted overnight from Day -1, treated with TNBS (17.5mg in 30% ethanol, Day 0), and imaged on Days 1,3,7 and 10. Two randomized groups received either sulphasalazine, a gold standard (SSZ, 300mg/kg n=9) or methylcellulose (1%, 10ml/kg, n=10) p.o. daily from Day -1. ***Imaging.*** Imaging used a 4.7T scanner (Bruker Biospec 47/40). We chose not to suppress peristalsis pharmacologically in case animal recovery for serial MRI was prejudiced. We used two respiration-triggered, fat-suppressed sequences covering a $(60mm)^3$ abdominal volume with 30 axial slices: T2-weighted (T2w, MSME, TE_{eff} 25.3ms, 256x192 matrix, 4 averages) and T1w contrast-enhanced (CE-MRI, FLASH, TE 2.16ms, FA 90°, 256x256 matrix); TR = 1/resp.rate ~1.1s. Five T1w images were acquired every ~5 minutes; contrast agent (Omniscan, 0.125 mmol/kg i.v., t=30s) was injected at the end of scan 3.
Analysis. Contrast agent injection caused a large signal intensity change in the colon wall between scans 3-4, varying only little before and after injection. The CE-MRI data were analysed using principal component analysis (PCA) yielding an average image (AVG, eigenimage 1), a map of contrast agent induced signal change (CE map, eigenimage 2), allowing the removal of uncorrelated motion-related intensity changes (eigenimages 3-5). The CE map fused with the AVG image in a colour-coded overlay (opacity proportional to CE map value) highlighted only tissues that received much CA (high perfusion, large extracellular space / blood volume) such as the bowel wall and mesentery. To discriminate between real perfusion changes and bowel motion coinciding with the CA injection, we quantified the signal variability (normalised to the respective pre-/post-injection baseline) assuming that a large variability will identify areas of motion. The normalised standard deviation (STDn) was obtained by splitting the time series into pre- and post-injection parts, dividing each part by the temporal average, and then calculating the temporal STD of the 5 normalized images. A STDn map was calculated, which determined the hue, going from yellow for STDn ≤ 0.15 to red for STDn ≥ 0.45.
To measure bowel wall thickness and amount of inflamed mesentery ("ColMes") we segmented the most affected image slice located in the volume 10-34mm proximal to the femoral head. Image processing employed newly developed and existing ImageJ [1] plugins and macros; plotting and statistics was done in MATLAB.

Results and Discussion: T2w and T1w MRI allowed colon wall delineation in most of the images slices; residual gut motion artefacts were caused by peristalsis. CE-MRI, analysed by PCA, clearly and reliably highlighted the ColMes. While most gut motion was captured in eigenimages 3-5 and hence eliminated, some potentially confounding motion that coincided with the CA injection was detected in the second PCA eigenimage. Using the STDn, image areas with motion could be quite reliably identified (red in Fig.1), making the segmentation more accurate. Based on the different image contrasts (Fig.2) it seems possible to distinguish the mucosal inner layer of the colon, the muscular colon wall and the surrounding inflamed mesentery.
Comparison of naïve and TNBS-treated rats revealed a large therapeutic window (Fig.3). The mean ColMes area increased following TNBS by 800% from normal values (Day 0), maximising by Day 3. SSZ, dosed from Day -1, did not inhibit the initial injury response to TNBS but it reduced the development of ColMes inflammation thereafter. Normal levels were not achieved by day 10 in this study, correlating with *ex vivo* histological findings, indicating that the inflammation is relatively long-lived.
In conclusion, the proposed methodology allows a quantitative estimation of inflammation-related changes in the colon wall and mesentery of the TNBS-treated rat, similar to those observed with MRI in mouse colitis [2,3]. While pre-dosing maximised the potential effect of the drug, we aim to employ the more realistic therapeutic strategy of treating colitis after initiation in future studies. This will allow the relative variability between animals to be controlled for and is a major advantage compared to conventional non-MRI studies using single ex vivo endpoints. Our results suggest that this technique could provide useful information for the evaluation of potential new treatments for IBD.

References: [1] Rasband WS (1997-2003) U.S. NIH, http://rsb.info.nih.gov/ij/. [2] Larsson AE *et al.* (2006) Inflamm Bowel Dis 12(6): 478-85. [3] Melgar S *et al.* (2007) Biochem Biophys Res Commun 355(4): 1102-7.

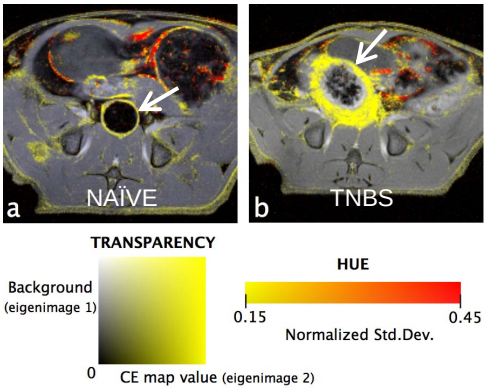

Fig. 1: Examples of a healthy (a) and inflamed colon (b). Colour-coded overlays (fusion of eigenimages 1 and 2) highlighting tissues with a CA-induced intensity increase (yellow) while correlated motion artefacts are identified (red) based on the normalized standard deviation.

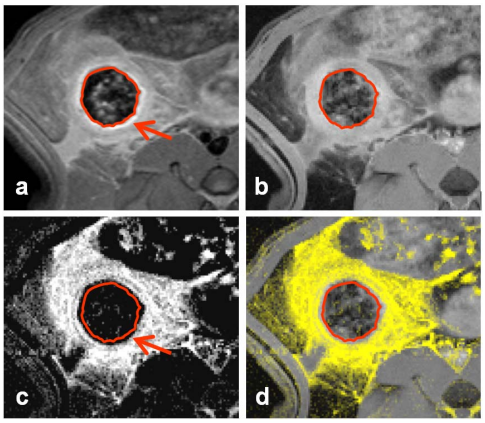

Fig. 2: Comparison of different image 'contrasts' for a severely inflamed colon. **a)** T2w image showing the thickened colon wall and the brighter mucosal layer; the red outline indicates for reference the mucosal surface; **b)** PCA eigenimage 1 ('average' of T1w image series) showing the colon wall with better contrast; **c)** PCA eigenimage 2 (map of CA-induced intensity increase which appears not to highlight the mucosal surface); **d)** color-coded overlay (yellow)

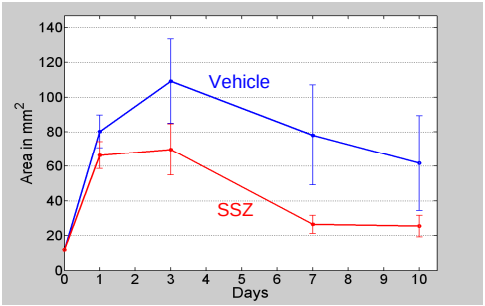

Fig. 3: Plots of cross-sectional area vs time, as derived from the segmentation of the tissues highlighted by the CA (yellow in Fig.2d). Mean area for all animals of the vehicle group (blue) and drug group (red) with standard errors (error bars).