High Field MR Imaging of Experimental Mouse Colitis

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Introduction

Inflammatory bowl disease (IBD) is characterized by an inflammatory condition of the colon such as that occurring in ulcerative colitis and Crohn's disease. Patients with inflammatory bowl disease have an increased risk for developing colon cancer. Clinically the diagnosis of IBD is based on laboratory and clinical findings including endoscopy and biopsies. Interluekin (IL)-10 is an anti-inflammatory and immune regulatory cytokine. IL-10 deficient mice (IL-$10^{-/-}$) when treated with the nonsteroidal anti-inflammatory drug, piroxicam, rapidly develop severe, transmural colitis that leads to onset of low and high grade dysplasia progressing to invasive colon cancer [1]. We have used high resolution MR imaging *ex vivo* to characterize the changes that occur in the colon of the IL-$10^{-/-}$ mouse treated with piroxicam. Increase in wall thickness, loss of colonic architecture, presence of edema, and regional changes in spin-lattice and spin-spin relaxation times of tissue water were observed in colitis. Using *in vivo* MR imaging, we have also examined the effects of treatment with anti-TNF mAb, a recommended therapy for patients suffering from IBD.

Methods

Healthy 4-week old wild-type and IL-$10^{-/-}$ B57BL/B6 mice were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were fed piroxicam for two weeks to induce colitis. They received 60mg piroxicam per 250gm of chow during the first week and 80mg of piroxicam per 250gm of chow the second week. One day after piroxicam treatment, mice were euthanized. The entire colon was excised, flushed thoroughly with PBS and immersion-fixed in 4% paraformaldehyde for two weeks before they were prepared for imaging. Prior to imaging, specimens were emptied of paraformaldehyde, rinsed with PBS and filled with Fomblin (Sigma Aldrich, St.Louis, MO). Colons were kept immersed in Fomblin during MR imaging. Fomblin prevents tissue drying and also reduces imaging artifacts arising from susceptibility gradients. High resolution images of normal and colitis colons (n=4) were acquired on a Bruker Avance 14.1T vertical axis microimager using a volume coil tuned to 600MHz. Gradient recalled echo 3D images were acquired with TR/TE 50msec/5msec and 78μm isotropic pixel size. T_1 and T_2 relaxation times were measured in 0.25mm thick slices by acquiring images with multiple TR and TE, respectively. Scanned colons were thoroughly washed in formaldehyde and routinely processed for H&E staining. Tissue was sliced to 6μm sections and stained.

Another group of piroxicam-treated IL-$10^{-/-}$ mice were injected daily with anti-TNF mAb (a murine chimeric Ab; Centocor) for three weeks starting on day 49 (seven weeks after initiation of piroxicam treatment). Multi-slice, spin echo images (TR/TE 1500msec/30msec) of anesthetized mice were acquired at 14.1T to measure colon wall thickness. Mice treated with a control antibody as well as B6 mice treated only with piroxicam were also imaged for comparison.

Results and Discussion

Figure 1. Comparison of high field MR image and histology of normal mouse colon. Left: Transverse slice from 3D GRE MR image at 14.1T with 78μm isotropic pixel size. Middle: H&E stain. Right: Volume rendered image generated from the 3D MR image shows the spiral pattern of colonic folds.

Images of a normal mouse colon (Figure 1) reveal that high field MR imaging is capable of spatial resolution that is high enough to visualize the structural details of colon that have been seen only with tissue histology. The three layers of colon, namely, muscular, submucosal and mucosal layers, as well as colonic folds can be distinguished in MR images.

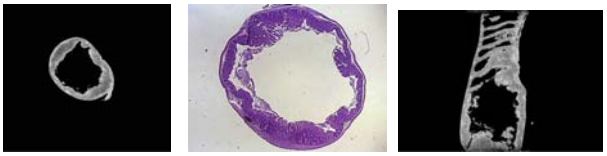

Figure 2. Comparison of high field MR image and histology of colitis in IL-$10^{-/-}$ mouse model. Left: Transverse slice from 3D GRE MR image at 14.1T with 78μm isotropic pixel size. Middle: H&E stain. Right: Sagittal slice from the 3D MR image shows damage to the spiral pattern of colonic folds in colitis.

The loss of normal colonic architecture caused by colitis is vividly seen in high resolution MR images (Figure 2) and confirmed by histology. Thickening of walls and distortion of the spiral pattern of colonic folds are prominent. All three layers are affected by inflammation with the muscular and the mucosal layers being more severely affected. From our MR images, the average wall thickness of piroxicam-treated IL-$10^{-/-}$ mouse colon is 0.90±0.15mm. Average wall thickness of normal mouse colon is only 0.20±0.02mm. Thus acute colitis causes a marked increase in the thickness of colon wall. Using diffusion-weighted imaging, we also detected the presence of edema in colitis. With b=1000s/mm^2, complete signal attenuation was seen in much of the mucosal layer surrounding the lumen. Several regions of the muscular layer also showed the presence of edema, which was less widespread in the intermediate submucosal layer of the colon. We further characterized mouse colon by measuring T_1 and T_2 relaxation times in each pixel of the MR image. Normal colon is spatially uniform in both T_1 (1.36±0.10s) and T_2 (16.01±1.72msec) whereas colitis has two distinct patterns for each relaxation time. T_1 and T_2 were 2.41±0.18s and 19.21±0.80msec, respectively, in regions surrounding the lumen as well as in the outer rim; they were 1.81±0.07s and 15.65±1.15msec in the region in between. The molecular basis of the observed changes in T_1 and T_2 relaxation times is the subject of ongoing investigation in our laboratory.

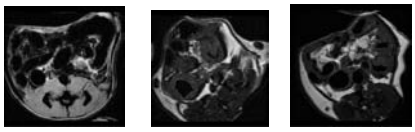

Figure 3. Effectiveness of anti-TNF Ab treatment on colitis assessed by MRI. Left: In vivo MR image of a control B6 mouse treated only with piroxicam shows normal colon wall. Middle: IL-$10^{-/-}$ mouse with colitis shows no reduction in wall thickness after treatment with a control Ab. Right: IL-$10^{-/-}$ mouse with colitis shows reduced wall thickness after treatment with anti-TNF mAb.

We used in vivo MR imaging to assess the effectiveness of anti-TNF mAb treatment in chronic mouse colitis induced by piroxicam feeding (Figure 3). Relative to mice treated with a control Ab (0.70-0.90mm), mice treated with anti-TNF Ab have significantly thinner colon wall (0.35-0.45mm). Piroxicam feed does not affect B6 mice as seen from lack of wall thickening (0.20mm).

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References

1. Berg DJ, et al. Gastroenterology, 2002; 123: 1527-1542.