Quantitative assessment of macrophage activity in inflammatory bowel disease using fluorine-19 MRI and the response to treatment

D. K. Kadayakkara[1,2], and E. T. Ahrens[1,2]
[1]Carnegie Mellon University, Pittsburgh, PA, United States, [2]Pittsburgh NMR Center for Biomedical Research, Pittsburgh, PA, United States

Introduction

Macrophages play an important role in the induction and progression of inflammatory bowel disease (IBD) (1). Longitudinal, non-invasive imaging of macrophage activity can be an important diagnostic tool in assessing disease progression and response to treatments. In this work, we characterize inflammatory burden in a mouse model of IBD using *in situ* macrophage labeling with perfluorocarbon emulsion (PFC) followed by ^{19}F MRI detection. ^{19}F signal was highly specific to macrophages in the colon, with no background signal, and linearly proportional to inflammation severity. We demonstrate that the ^{19}F signal, and hence macrophage activity in the gut, decreased in mice treated prophylactically with cyclosporine A (CsA), but increased in mice treated with dexamethasone. Results were validated using *ex vivo* MR microscopy, confocal microscopy, histology, and qRT PCR.

Methods

IBD was induced in IL10$^{-/-}$ mice (n=16) by feeding piroxicam-doped chow for 14 days. Five of these mice received dexamethasone (1. 2 mg/kg i.p daily for 14 days) and five of them received CsA (10 mg/kg i.p daily for 14 days). PFC emulsion (VS-1000H, Celsense, Pittsburgh, PA) containing a trace amount of DiI fluorophore in the surfactant was injected i.v. (0.2 mL) via the tail vein. Anesthetized mice were imaged two days after PFC injection. Multi-slice, ^{1}H MRI data were acquired at 11.7 T through the abdomen, along with co-registered ^{19}F images. ^{19}F signal intensity in the colon was quantified relative to an external PFC reference tube using Voxel Tracker software (Celsense). Excised colon tissues were also imaged *ex vivo* using MR microscopy. Colon tissues were subjected to H&E histology, immunohistochemistry to look at macrophages (F4/80), neutrophils, monocytes (Ly6C) and endothelial cells (CD31), and RNA was extracted to measure macrophage load using qRT PCR.

Results and conclusions

Thickening of the colon wall was observed in ^{1}H images, and patchy ^{19}F signals were observed (Fig. 1a). H&E staining displayed pancolitis with heavy mononuclear cell infiltration. Immunofluorescence of colon tissues showed that PFC was localized exclusively within macrophage (Fig. 1b). The qRT PCR revealed a linear correlation between macrophage RNA and ^{19}F signal in the same tissue samples (R=0.65, p=0.03) (Fig. 1c). The colon ^{19}F signal was reduced in CsA treated animals and increased in dexamethasone treated animals in all regions of the colon (Fig. 1d), reflecting changes in macrophage burden. The ^{19}F data was validated by histology scores in the colon. Our results demonstrate that ^{19}F MRI is an effective method to quantitatively measure macrophage activity *in vivo* and assess treatment response in IBD.

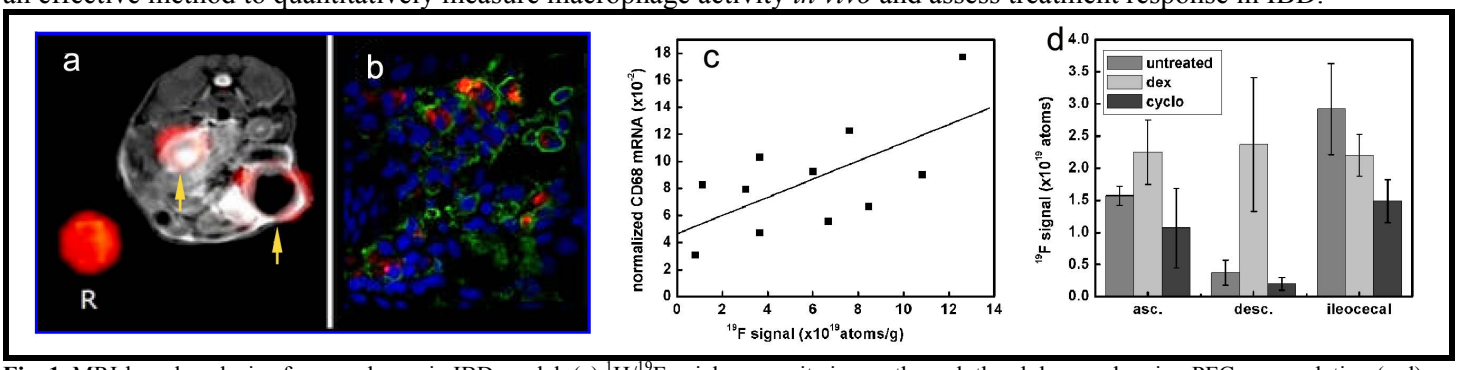

Fig. 1. MRI-based analysis of macrophages in IBD model. (a) ^{1}H/^{19}F axial composite image through the abdomen showing PFC accumulation (red) in ascending and descending colon walls (arrows). R represents the reference tube containing PFC. (b) Immunohistochemistry of colon tissue where macrophages engulfing PFC-DiI (F4/80 macrophage is green, PFC-DiI is red, nuclei are blue). (c) qRT PCR results showing linear correlation plot between ^{19}F signal and macrophage mRNA in the same colon segments (R=0.65, p=0.03). Expression of macrophage mRNA CD68 is normalized with expression of mRNA of a house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). (d) Quantitative analysis shows an increase in ^{19}F signal in dexamethasone treated animals (n=5) and a decrease in cyclosporine treated animals (n=4) in ascending colon (asc.), descending colon (desc.) and ileocecal region of the colon compared to untreated controls (n=5).

References

1. Watanabe N. et al. (2003) *Dig Dis Sci* 48(2): 408-414.

Acknowledgements

We thank Lisa Pusateri for technical assistance and Dr. Won-Bin Young for helpful discussion. This work is funded by NIH R01-CA134633, R01-EB003453, P41-EB001977.