Magnetic resonance texture analysis is superior to magnetization transfer imaging for longitudinal assessment of fibrosis

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Abstract

A common and untreatable complication of Crohn's disease (CD) is the development of intestinal fibrosis for which no adequate noninvasive tools for fibrosis progression monitoring exist. For this purpose, we assessed the performance of magnetization transfer (MT) magnetic resonance imaging (MRI) and texture analysis (TA) on T2-weighted images by correlating them with histology in a fibrosis mouse model. MT-MRI and TA performance was validated during antifibrotic therapy in mice and on MR enterography images of CD patients undergoing bowel surgery. The MT-MRI and TA entropy correlated with histological fibrosis (r = .85 and .81, respectively). However, TA entropy was superior to MT-MRI for quantifying fibrosis progression during coexisting inflammation (linear regression R^2 = .93). TA entropy could efficiently monitor anti-fibrotic therapy efficacy and correlated with the grade of intestinal fibrosis in CD. In conclusion, MT-MRI and TA entropy are highly promising surrogate markers of intestinal fibrosis, but TA entropy performed better in the presence of concomitant inflammation.

Introduction

Crohn's disease (CD) is a multifactorial disease characterized by distinct clinical phenotypes and can evolve from an uncomplicated disease at diagnosis to a stricturing or penetrating disease over time. A stricturing disease results from excessive scar tissue accumulation, leading to clinical stenosis over time. Reported incidence rates range from 26% at diagnosis to 48% within 5 years and up to 70% within 10 years after diagnosis.[1, 2] However, precise prospective data describing the progression of structural bowel damage is missing, primarily because the stricturing disease is ill-defined.[1, 3, 4] Despite major progress in controlling inflammation in patients with CD using an increasing array of approved drugs, the drugs do not seem to have a clear effect on the progression of the structural damage to the bowel. In line with this observation, growing evidence indicates that strictures can also develop from inflammation-independent processes.[5] In addition, the risk and development speed of fibrostenosis are highly variable.[1, 4, 6]

Although no drugs have been approved to prevent fibrosis development in CD, safe and efficient antifibrotic therapies have been developed for other organs, and such agents may also become available for patients with CD.[7, 8, 9, 10] However, a consistent monitoring approach for intestinal strictures is lacking, impeding the development of clinical studies. As stricturing bowel segments contain variable proportions of inflammatory and fibrotic tissue, fibrosis quantification is challenging.[11, 12] However, differentiating fibrotic strictures from inflammatory narrowing of the bowel lumen is clinically important because symptomatic fibrotic strictures are currently an indication for endoscopic balloon dilatation or surgery, while inflammatory strictures may benefit from conventional anti-inflammatory therapy. Endoscopic assessment of fibrosis in strictures is inefficient because of the transmural nature of the lesions in CD. The value of the histological analysis of bowel wall biopsies to determine the grade of fibrosis is also limited due to the superficial nature of the samples and the high risk of sampling error. The gold standard for assessing fibrosis is the histopathology of full-thickness biopsies or surgically resected strictures, which is not useful during clinical trials.[13] Conventional cross-sectional imaging modalities, such as

ultrasound, computed tomography (CT) enterography, and magnetic resonance enterography (MRE), have high accuracy for detecting bowel inflammation. However, these techniques cannot accurately measure the amount of fibrosis in a stricture.[3, 11, 14, 15]

Magnetic resonance imaging (MRI) has gained broad acceptance as the reference technique for assessing the disease extent and activity in CD, especially due to its lack of radiation exposure.[15] Routinely used magnetic resonance (MR) sequences have also been used to quantify intestinal fibrosis in CD, but inconsistent data have been reported, as superimposed inflammation can mask transmural fibrosis.[11, 14, 16] Emerging new imaging modalities, such as magnetization transfer (MT) MRI and texture analysis (TA), demonstrate great potential to evaluate intestinal strictures in CD.[17, 18, 19, 20, 21, 22, 23, 24, 25, 26] In addition, MT-MRI is a noninvasive imaging method that enables the indirect measurement of large, immobile macromolecules, such as collagen, in an aqueous physiological environment. Further, MT-MRI has been reported to accurately detect and quantify intestinal fibrosis in animal (rat) studies and small cohort human studies.[17, 18, 19, 20, 21, 22, 23] This technique seems sensitive to changes in bowel fibrosis and not to changes in inflammation; however, most reported studies have included one specific timepoint with no longitudinal assessment.[17, 20, 21, 23]

The TA is a post-processing technique applied to cross-sectional images to characterize tissue composition. It quantitatively assesses patterns of MRI-signal intensity distribution within a selected image region, producing textural parameters that provide indirect tissue heterogeneity information not visible to the human eye.[27] Moreover, TA has been successfully applied to cross-sectional cancer imaging and may aid in prognosis and treatment response.[28, 29] The use of TA on noncontrast MR images in intestinal fibrosis has not yet been studied. Furthermore, the ability of TA to detect changes in intestinal fibrosis over time also remains to be investigated.

Results

Magnetization transfer ratio and texture analysis entropy detect fibrosis during subclinical inflammation. Bowel inflammation was induced through cyclic DSS administration, resulting in relapsing and remitting disease activity (Fig. 3A) and a progressive increase in histopathological fibrosis scores at Weeks 1, 6, and 9 compared to healthy control mice ($F(3,14)=[41.76]$, $p = .0217$, $p = .0006$, and $p < .0001$, respectively; Figure, 3B). Histological inflammation was observed in DSS-treated mice sacrificed at Week 1 ($F(3,14)=[20.80]$, $p < .0001$), whereas inflammation was only marginal in samples collected at Weeks 6 and 9 (Fig. 3C). Histopathologic scores and imaging parameters acquired during each recovery phase were plotted to assess the performance of MTR and TA as surrogate markers for fibrosis in the presence of subclinical inflammation. The MTR values were strongly correlated with the histopathological fibrosis scores ($r = .85$; $p = .0005$; Fig. 3D). Of the three textural parameters analyzed by TA (skewness, kurtosis, and entropy), only entropy correlated well with the fibrosis scores ($r = .81$; $p = .0011$; Fig. 3E).

Texture analysis entropy, but not the magnetization transfer ratio (MTR), enables monitoring fibrosis with a coexisting inflammation. In the presence of inflammation, TA entropy outperformed the MTR in quantifying the increase in fibrotic tissue after each DSS administration (Week 1 vs. Week 4: Δ *mean* = 0.338, $p = .0010$ for TA entropy and Δ *mean* = -0.065, $p = .1070$ for the MTR; Week 1 vs. Week 7: Δ *mean* = 0.471, $p = .0040$ for TA entropy and Δ *mean* = 0.051, $p = .2710$ for the MTR; Fig. 4A). During the sequential recovery phases with diminishing inflammation and progressive build-up of fibrosis, only the MTR and TA entropy at Week 9 were significantly higher compared to Week 3 (Δ *mean* = 0.208, $p < .0001$ and Δ *mean* = 0.366, $p = .0050$, respectively), indicating that only well-established fibrosis during recovery is detectable by both MRI parameters (Fig. 4B). When combining multiple scan moments, including baseline scans, linear regression indicated that the MTR could not capture the steady increase in fibrotic tissue in the presence of inflammation throughout the experiment ($p_{slope} = 0.7780$, $R^2 = .01$, Fig. 4C). In contrast, the TA entropy demonstrated excellent performance for monitoring fibrosis progression during periods of active inflammation alternated with recovery periods ($p_{slope} = 0.0004$, $R^2 = .93$, Fig. 4D). The MTR values were unexpectedly high at the baseline and consistently lower in the presence of inflammation at each cycle of DSS (Week 0 vs. Week 1: Δ *mean* = -0.598, $p < .0001$; Week 3 vs. Week 4: Δ *mean* = -0.205, $p < .0001$; and Week 6 vs. Week 7: Δ *mean* = -0.0910, $p = .1010$).

IncrEn represents a surrogate marker for assessing antifibrotic therapy response. Next, we aimed to determine the performance of MTR and TA entropy in monitoring fibrosis in mice treated with a well-established antifibrotic compound.[30] The DAI demonstrated a similarly proficient induction of bowel inflammation throughout the consecutive DSS cycles between the three groups of mice (healthy control group, placebo group, and treated group), indicating that the antifibrotic compound did not alter intestinal inflammation induced by DSS (Fig. 5A). At Week 9, placebo mice exhibited significantly higher histopathological fibrosis compared with the treated mice ($F(2,33)=[72.46]$, $p < .0001$), confirming the effectiveness of the treatment (Fig. 5B). This antifibrotic effect was associated with significantly lower colonic tissue levels of the pro-fibrotic cytokines IL-6 and TGFß1 ($F(2,30)=[3.189]$, $p = .0341$ and $F(2,32)=[7.676]$, $p = .0171$, respectively; Fig. 5C).

The placebo and healthy control mice exhibited similar MRI parameter values in line with the first experiment. Nonetheless, with the inclusion of the antifibrotic treatment group, a negative correlation was found at Week 9 between the MTR values and fibrosis grade ($r = -.90$; $p < .0001$; Fig. 6A). The combined MR data from both experiments demonstrated that a rise in the MTR value could theoretically indicate bowel tissue receiving treatment that develops toward normal tissue or diseased bowel tissue in which fibrosis is accumulating (Fig. 6B). Because of these unexpected findings and the inflammation-dependent drop in the MTR observed in the first experiment, we further investigated the influence of inflammation on the MTR values. To this end, the DAI scores were plotted against the MTR values from all scans collected in the two experiments, resulting in a significant negative correlation ($r = -.68$; $p < .0001$; Fig. 6C). This result confirmed that the coexisting inflammation substantially affects MTR acquisition. In contrast to the heterogeneous MTR data, a strong positive correlation between fibrosis and TA entropy was

confirmed, using the increase in entropy compared to the baseline entropy (IncrEn; $r = .84$; $p < .0001$; Fig. 6D).

The MTR values are influenced by inflammation; thus, we focused on the TA entropy to evaluate the effect of the antifibrotic therapy. A progressive increase in IncrEn was observed in the placebo-treated group (baseline vs. Week 3: Δ *mean* = 0.079, $p < .0001$; Week 3 vs. Week 6: Δ *mean* = 0.039, $p < .05$; and Week 6 vs. Week 9: Δ *mean* = 0.064, $p < .0001$). In contrast, only a significant but marginal change was noted between the baseline and Week 3 in the compound-treated group (Δ *mean* = 0.037, $p < .05$). At the endpoint scan (Week 9), IncrEn values were significantly higher in the placebo-treated mice compared with the compound-treated mice (Δ *mean* = 0.128, $p < .0001$; Fig. 5D).

Proof of concept for texture analysis entropy to quantify fibrosis in patients with Crohn's disease. The T2-weighted MRE images of five patients with CD collected before ileocecal resection were retrospectively analyzed to provide proof of concept for translating the findings in patients with CD. The demographic and clinical characteristics of the included patients are summarized in Supplementary Table 3. Using the histologic evaluation of the resected specimens (i.e., terminal ileum), one bowel segment was categorized as predominantly inflammatory, two segments as mixed inflammatory fibrotic, and two segments as predominantly fibrotic. The NormEn was 1.29 for the inflammatory bowel segment, 1.40 and 1.48 for the mixed segments, and 1.73 and 1.90 for the fibrotic segments, indicating that an increase in TA entropy can serve as a parameter to detect and grade intestinal fibrosis in patients with CD (Fig. 7).

Discussion

This preclinical study demonstrated that two novel imaging tools, MT-MRI and TA, can accurately detect and quantify intestinal fibrosis in a chronic colitis model. We found that only TA entropy can identify fibrosis in concomitant inflammation, as in mixed CD strictures. These mixed-type lesions are the most difficult by far to assess on routinely used MR sequences because the coexistence of both inflammation and fibrosis complicates stricture characterization. Therefore, stricture assessment using TA entropy may be especially useful to detect early intestinal fibrosis and monitoring fibrosis in patients with CD, facilitating decisions on individualized treatment strategies.

This method represents a significant improvement beyond previous studies investigating other quantitative MRI techniques, such as bowel wall perfusion or diffusion-weighted MRI, which have limitations in detecting the fibrotic component within mixed strictures.[31, 32, 33] In addition, these findings are consistent with the only previously published study focusing on the use of TA in intestinal fibrosis.[26] In this study, TA was performed on gadolinium-enhanced T1-weighted MRE images in a pediatric population, demonstrating that TA entropy can accurately distinguish strictures with fibrosis (mixed or purely fibrotic) from inflammatory strictures.[26] However, recent concerns regarding gadolinium tissue deposition favor the use of noncontrast MRI.[34] Furthermore, gadolinium contrast use implies an additional cost and is more time-consuming than standard T2-weighted MRI. Therefore, we used T2-

weighted MR images for textural image analysis in this study. To our knowledge, TA of T2-weighted MR images in intestinal fibrosis has not yet been investigated.

The results suggest that MTR is sensitive to intestinal inflammation and is thus unsuitable for fibrosis monitoring in an inflammatory context, contradicting previous reports. Adler *et al.* compared MT images in early-phase intestinal inflammation with late-phase fibrosis in a rat model and found increasing MTR values correlating with an increase in histologic fibrosis and a decrease in histologic inflammation, suggesting that MTR is sensitive to changes in fibrosis, but not to tissue inflammation.[17] Nonetheless, no baseline values were reported. More importantly, inflammation was induced by a single trigger; therefore, we suspect that inflammation could not have influenced the MTR measurements in this particular experimental setup. Dillman *et al.* demonstrated that MTR can distinguish between predominantly inflammatory and mixed inflammatory-fibrotic bowel tissue in a chronic colitis rat model.[20] Again, no baseline scan parameters were reported, and only mixed strictures were investigated.

Conflicting results have been reported in human studies. Li *et al.* analyzed MTR data for intestinal strictures in 31 patients with CD using surgical histopathology as the reference standard, finding that the MTR can accurately detect fibrosis and is not influenced by the presence of inflammation in the same intestinal stricture.[21] However, Fang *et al.* found that the histological inflammation score of intestinal strictures in 27 patients with CD weakly correlated with the MTR, indicating some, albeit weak, association exists between inflammation and the MTR.[23]

Furthermore, TA entropy was sufficiently sensitive to detect the early development of intestinal fibrosis and accumulation of fibrosis over time, whereas MT-MRI could only identify late-phase bowel fibrosis. This outcome is partially in line with the study by Adler *et al.*, who used MT-MRI to detect changes in bowel wall fibrosis over time in a rat model using subserosal injections of peptidoglycan-polysaccharide in the cecum to cause inflammation and subsequent fibrosis.[17] Weekly MT imaging was performed for 28 days after injection. A progressive increase in MTR was found; however, no significant difference exists in the MTR between the study and control animals until fibrosis was well-established, confirming the results.

Finally, we demonstrated that TA entropy could be used as a noninvasive tool in monitoring the response to antifibrotic treatment. This result may have a high clinical diagnostic value, as recent studies have shown that early intestinal fibrosis in CD can be reversed.[13, 35] To our knowledge, this is the first study to identify the value of this technique for in vivo assessment of the effects of antifibrotic therapy on bowel wall fibrosis. This outcome may be a major finding, as the lack of reliable and noninvasive diagnostic tools currently hinders antifibrotic therapeutic clinical trials in CD.

Although the results reveal that both modalities, MT-MRI and TA, can detect and quantify intestinal fibrosis, we believe that TA entropy holds tremendous promise to become a reliable parameter for quantitative assessment and longitudinal monitoring of intestinal fibrosis and should be explored further. First, TA entropy outperforms MTR in detecting the fibrotic component in mixed inflammatory-fibrotic

bowel tissue, enables early detection of fibrosis development, and can monitor bowel fibrosis progression. Second, the proof-of-concept data suggest that TA entropy can also determine the extent of fibrosis in human bowel segments. Third, TA entropy has the potential to serve as a tool for antifibrotic treatment response assessment. Finally, a general advantage of TA is its intrinsic quantitative nature, lending itself well to artificial intelligence algorithms that can automatically extract imaging features from medical image data undiscernible to the human eye. Such algorithms could significantly improve the radiologist's interpretation of patient images and offer significant prospects to derive quantitative imaging markers of disease activity and therapy response. Recently, Li *et al.* proposed a CT enterography-based radiomics model for fibrosis quantification in patients with CD, but no MR-based model has yet been developed.[36]

Methods

Part of the methods are described in a supplementary file.

Patient and public involvement. Patients were not actively consulted for this study.

Ethics. The Institutional Review Board of Ghent University approved the animal studies (ECD/19–68 and ECD/20-67aanv). Mice were housed in the animal facility at Ghent University Hospital (Ghent, Belgium) according to the institutional animal healthcare guidelines. The study using patient samples was approved by the Ethics Committee of Ghent University Hospital (EC/2018/1493, Belgian study registration number B670201838339), and written informed consent was obtained from all participants.

Induction of gut fibrosis in mice. Seven-week-old male C57BL/6J mice (Janvier Labs, Le-Genest-Saint-Isle, France) were housed in open cages in a temperature-controlled room at 20°C with a dark-light cycle of 12h. All interventions occurred during the light cycle. Animals had free access to water and commercial chow (mouse maintenance chow, Carfil Labofood, Belgium) ad libitum, and no fasting periods were implemented. In addition, 2.5% dextran sulfate sodium (DSS, MW = 36,000 to 50,000; MP Biomedicals, Illkirch, France) was supplemented to the drinking water for seven consecutive days to induce intestinal fibrosis followed by a two-week recovery period during which mice received normal drinking water. This cycle was repeated twice. The induction of the intestinal inflammation caused by DSS was followed up using the Disease Activity Index (DAI), a combinational score of the weight evolution, stool consistency, and presence of blood in the stool.[37]

In the first experiment, 16 mice were subjected to DSS administration, and two control mice were included, receiving normal drinking water. The control mice underwent an MR examination at the baseline and endpoint (Weeks 0 and 9). Mice receiving DSS treatment were subsequently scanned after every DSS-induced inflammation peak (i.e., Weeks 1, 4, and 7) and after each two-week recovery period (Weeks 3, 6, and 9; Fig. 1A). Intermediate sampling occurred after scanning at Week 1 (n = 5 out of 16), Week 6 (n = 5 out of the remaining 11) and Week 9 (n = 6) for histological inflammation and fibrosis assessment. Control animals (n = 2) were sacrificed at Week 9. We first correlated the histopathological data with MRI

parameters during recovery to assess the MRI parameters as a surrogate marker for fibrosis (Weeks 6 and 9). Next, we aimed to evaluate the correlating parameters for the longitudinal assessment of fibrosis development during chronic inflammation. To this end, scan parameters from all MRI scans were analyzed (Fig. 1A).

In the second experiment, we investigated the application of the best performing MRI parameter based on the results from the first experiment as a surrogate marker for antifibrotic therapy response. The mice were randomized into three groups for this experiment, each containing 12 animals (Fig. 1B). Control mice were scanned at the baseline and end of the experiment (Week 9). Intestinal fibrosis was induced in the remaining 24 mice, and these animals underwent MRI at the baseline and after each two-week recovery period (Weeks 3, 6, and 9). The baseline scan was included to allow a more accurate grouped comparison of the changes in MR parameters. The DSS-treated mice received REDX08397 daily (p.o. gavage, 10 mg/kg; Redx, Macclesfield, UK) suspended in an aqueous 0.5% (hydroxypropyl)methylcellulose solution (Sigma-Aldrich, MO, USA) or placebo (aqueous 0.5% (hydroxypropyl)methylcellulose solution).[30] All mice were sacrificed after the final MR examination (Week 9).

Magnetic resonance imaging protocol. All details of the scanning protocol are available in Supplementary Table 1. In addition, MRI was performed on a 7 Tesla MRI scanner (PharmaScan, Bruker Biospin, Ettlingen, Germany). Axial T2-weighted images were obtained in each mouse. The MT imaging was acquired using two gradient-echo data sets with and without applying an off-resonance prepulse (frequency offset 2 kHz).

Magnetic resonance imaging analysis. Axial T2-weighted images were reviewed, and the most distal part of the distal colon was identified in each mouse. Two readers manually drew a region of interest (ROI) in consensus (a radiologist with 11 years of experience (IDK) and a research fellow with 3 years of experience (SB)) in the distal colon in an area where the bowel wall was well defined. The full thickness and entire circumference of the bowel wall was included within the ROI. Care was taken to exclude any intraluminal or mesenteric tissue. Once defined on the T2-weighted image, the ROI was copied and applied to the corresponding MT-image map (Fig. 2). If necessary, to account for bowel motion, minor adjustments for the position or size of the ROI were made when the ROI was copied.

The MT value was calculated using the formula $(MT0 - MTsat) / MT0$, where MT_{sat} and MT_0 refer to the signal intensities acquired with and without the off-resonance prepulse saturation, respectively. The MT-image maps were generated using open-source image processing software (ImageJ, US National Institutes of Health, Bethesda, Maryland, USA). The MT of the bowel wall was divided by the MT of the paraspinal muscle at the same slice in each mouse to obtain a normalized MT ratio (MTR) to minimize individual variation.

Axial T2-weighted MR images were uploaded into commercially available research software (TexRAD, Feedback Plc, Cambridge, UK). The TA with a filtration-histogram technique was performed within the

selected ROI using a previously published methodology.[27] Filtration extracted and enhanced image features of different sizes (radii from 0 to 6 mm) within the ROI before the subsequent histogram quantification. A spatial scale filter of 2 mm was chosen for this study, highlighting the fine textural features. The heterogeneity within the ROI was quantified, and three textural parameters were obtained: kurtosis (pointiness of the pixel distribution), skewness (asymmetry of the pixel distribution), and entropy (inhomogeneity of the pixel distribution). These heterogeneity features have been previously described.[27]

Regarding the entropy, we calculated the incremental entropy (IncrEn) as a relative measurement to allow a more appropriate comparison between different experiments. This parameter represents the increase in entropy in the pathologic bowel over the normal bowel. This value was calculated in Experiment 1 by dividing the entropy in each mouse by the mean entropy of the two control animals at the baseline. In Experiment 2, IncrEn was obtained by dividing the entropy in each mouse by its entropy value at the baseline.

Proof-of-concept translation of magnetic resonance/texture analysis findings to Crohn's disease in humans. The mean interval between MRI and surgery of the patients was 35 days. Region-by-region correlations between surgical specimens and MRE were conducted by an experienced radiologist (IDK) who was blinded to the clinical and histopathological data. Matching locations between the resected bowel segments and MRE images were identified using anatomic landmarks (surgically resected margins and ileocecal valve) or gross lesions (bowel fistula). The most stenotic areas were selected for histopathological assessment and MRE correlation, and one specimen per patient was obtained. Routine clinical assessments require only a conventional MRE; thus, no MT images were available, and only TA was feasible. The TA was performed by placing an ROI on the pathologic small bowel wall on the axial T2-weighted images in each patient ($n = 5$) and in a normal-appearing small bowel ($n = 5$) to calculate the normalized entropy ($NormEn = entropy_{\ pathologic\ bowel} / entropy_{\ normal\ bowel}$).

Statistical analysis. This study was codesigned and analyzed by an expert statistician (Biostatistics Unit, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium). The statistical analyses were performed in SPSS (v. 27; IBM, Armonk, NY) and all tests were two-tailed. A linear mixed model assessed differences in body weight and DAI progression. The Pearson correlation test was applied to explore the relationship between MRI parameters and pathophysiological data. A one-way analysis of variance was used when comparing the normally distributed data between groups, applying Dunnett's multiple comparison correction (F values and adjusted p-values reported). Linear regression and a mixed model analysis were used to evaluate the MRI parameters. The Akaike information criterion was consulted to select the best covariance structure to perform a mixed model analysis when comparing parameters between different scan timepoints. The *p*-values from the mixed model analysis were corrected for multiple comparisons using the more stringent 99% confidence interval.

DATA AVAILABILITY

No data, methods or study materials will be made available to other researchers.

Declarations

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Author contributions

Study concept and design: SB, IDK, PH, MDV, and DL

Acquisition of data and analysis: SB, IDK, and SVW

Writing of the manuscript: SB and IDK

Critical revision of the manuscript: SB, IDK, LD, PB, SVW, PH, MDV, GV, and DL

Competing Interests

PB is an employee of Redx Pharma Lpc. Other authors declare that they have no competing interests.

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Figures

Figure 1

Overview of the study design. Gut fibrosis was induced in mice by administering dextran sodium sulfate (DSS) to the drinking water for one week (acute phase), followed by two weeks with normal drinking water (recovery phase). This cycle was repeated twice. (A) In the first experiment, magnetic resonance imaging (MRI) scans were performed after every DSS week and after the recovery period in each cycle, capturing inflammatory (Week 1), mixed (Weeks 4 and 7), and predominantly fibrotic bowels (Weeks 3, 6, and 9). Mice were sacrificed for pathohistological examination at three points (†). (B) In the second experiment, all mice were scanned at the baseline (Week 0) and after each two-week recovery period (Weeks 3, 6, and 9) to monitor the effect of antifibrotic therapy. After the final MRI scan, all mice were sacrificed for histopathological assessment (†).

Figure 2

Example magnetic resonance (MR) images obtained from a mouse. Gut fibrosis was induced in mice by administering dextran sodium sulfate to the drinking water for one week, followed by a period of two weeks with normal drinking water. This cycle was repeated twice. (A) Axial T2-weighted MR image of the distal colon at Week 9 and (B) the corresponding magnetization transfer map demonstrating the positioning of the region of interest.

Figure 3

Correlation between fibrosis on histopathology, magnetization transfer ratio (MTR) and texture analysis (TA) entropy in the dextran sodium sulfate (DSS) mouse model. Gut fibrosis was induced in mice by administering DSS to the drinking water for one week, followed by two weeks with normal drinking water.

This cycle was repeated twice. (A) Disease Activity Index (DAI) score throughout the experiment. (B) Inflammation scores and (C) intestinal fibrosis scores for healthy control mice (n = 2) and DSS-treated mice sacrificed at Weeks 1, 6, and 9 (n = 5, 5, and 6, respectively). (D) Correlation of MTR with histopathology and (E) TA entropy with histopathology using the fibrosis scores from each recovery phase (n = 11). AU: arbitrary units; *p < .05; ***p < .001; ****p < .0001; Data in A, B, and C are represented as the mean ± the standard error of the mean.

Figure 4

Magnetic resonance (MR) parameters during dextran sulfate sodium (DSS) induced inflammation and the following recovery. (A) Magnetization transfer ratio (MTR) and texture analysis (TA) entropy values acquired during the acute inflammatory phase of each DSS cycle (Weeks 1, 4, and 7 (n = 16, 11, and 6, respectively)). (B) MTR and TA entropy values acquired during the recovery period for each DSS cycle (Weeks 3, 6, and 9 (n = 11, 11, and 6, respectively)). (C) MTR values and (D) TA entropy values throughout the experiment with the linear regression trend line (Week 0 are healthy controls (n = 2)). AU: arbitrary units; * p < .05, **p < .01, and ***p < .001. Data are represented as the mean ± the standard error of the mean.

Figure 5

Texture analysis (TA) entropy represents a novel tool for quantifying response to antifibrotic therapy (REDX08937) in chronic dextran sulfate sodium (DSS) colitis. Gut fibrosis was induced in mice by administering DSS to the drinking water for one week, followed by two weeks with normal drinking water. This cycle was repeated twice. Mice were treated with an antifibrotic compound (REDX08937) or placebo (n = 12 each). (A) Disease Activity Index (DAI) scores throughout the experiment. (B) Histopathological fibrosis analysis and (C) interleukin-6 and transforming growth factor ß1 protein levels in the colon of healthy, REDX08937-treated, and placebo-treated DSS mice at Week 9. *p < .05; **p < .01; ****p < .0001. Data are represented as the mean ± the standard error of the mean; n= 12 for all groups.

Figure 6

Correlation between histopathology, magnetization transfer ratio (MTR), and texture analysis (TA) entropy in the dextran sulfate sodium (DSS) mouse model treated with an antifibrotic compound. (A) Correlation of the MTR with histopathology of fibrosis at Week 9. (B) Combined MTR data from the first and second experiments plotted in the function of fibrosis scores determined after scarification at Week 6 (Experiment 1) and Week 9 (Experiments 1 and 2). (C) Combined MTR data from the first and second experiments

plotted against the Disease Activity Index (DAI) scores. (D) Correlation between histopathology and incremental entropy (IncrEn) for the second experiment. (E) Evolution of grouped IncrEn values (n = 12 per scan moment per group) for magnetic resonance (MR) scans performed in healthy, REDX08937-treated, and placebo-treated DSS mice after each recovery phase. AU: arbitrary units. Data are represented as the mean ± the standard error of the mean.

Figure 7

Texture analysis (TA) entropy as a surrogate marker of fibrosis in patients with Crohn's disease. (A) Axial T2-weighted magnetic resonance (MR) images with region-of-interest placement in the affected terminal ileum (TI) and normal-appearing small bowel (SB) are depicted for patients with three different histologic types (i.e., active inflammation without fibrosis (left panel), active inflammation and fibrosis (mid panel), and fibrosis without active inflammation (right panel)). (B) Entropy was analyzed by TA in MR enterography (MRE) images of five patients (P1-P5), revealing increasing normalized entropy values (NormEn) when fibrotic tissue accumulates in the bowel wall, whereas these values in the normal SB remain at a similar level in all patients. AU: arbitrary units.

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