Supplementary Methods

Histopathologic Analysis of Animal Tissue

Two samples from proximal and distal colon were collected immediately after the UEI procedure. Samples were fixed in 10% buffered formalin, paraffin embedded, underwent hematoxylin and eosin, Masson's trichrome, and α-smooth muscle actin (α-SMA) staining, and were assessed by pathologists (D.M., B.M.) in a blinded fashion. Parameters assessed included mucosal, submucosal, and muscularis mucosa thickening, degree of trichrome deposition, degree and type of cellular infiltrate, presence of mucosal ulceration and evidence of necrosis, and overall architectural distortion. After review of all specimens, a qualitative combination of factors yielded two pathology scores for overall inflammatory and overall fibrostenotic characteristics (scale: 0 = none, 1 = mild, 2 = moderate, 3 = severe).

Immunoblotting

Immunoblotting was utilized for the detection of α-SMA. Whole tissue was pulverized under liquid nitrogen and lysed in ice-cold RIPA buffer with a cocktail of proteinase inhibitors (Roche, Indianapolis, IN). Protein content was determined using a modified Bradford assay kit (Bio-Rad, Hercules, CA). Total protein was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Amersham Biosciences, Piscataway, NJ).

Membranes were blocked in 5% milk solution for 1 hour at room temperature or overnight at 4°C. α-SMA was detected by incubating the membrane overnight at 4°C with mouse antihuman monoclonal antibody (Sigma, St. Louis, MO) at 1:5000 dilution in 5% milk/TBST. As a loading control, a mouse antibody for GAPDH (Chemicon, Temecula, CA) was used. Secondary antibody anti-mouse IgG HRP (Amersham) was incubated for 1 hour at room temperature and the signal was detected by the Pierce detection system (Rockford, IL).

Ultrasound Image Processing for UEI Strain Estimates

Two-dimensional sections of distal (treated) and proximal (untreated) colon regions of interest (ROI) were identified by a clinical ultrasound expert (J.M.R.) in the original B-scan images. Strain estimates were generated off-line by processing RF data using phase-sensitive, correlation-based, 2-dimensional speckle tracking algorithms which calculated the in-plane frame-to-frame axial and lateral displacement.[1,2] The normalized strains were determined from the mean strain developed in the ROI (where correlation coefficient of 2D-speckle tracking is >0.9) divided by the magnitude of the applied mean strain averaged over imaging area (excluding ROI) where correlation coefficient of 2D-speckle tracking is >0.9 within the linear tissue elastic region of $<15\%$ of strain. Negative strain values represent tissue compression and positive values reflect expansion. Strain ratio is the average strain of the distal colon divided by the average strain of the proximal colon.

Direct Mechanical Measurements

Tissue sections from distal and proximal colon were cut into 1×1-cm squares for immediate, direct mechanical measurement using an elastometer (Micro-Elastometer, Artann Laboratories, West Trenton, NJ). The thickness of the samples ranges from 1.0 to 4.0 mm. The elastometer is composed of a pressure-sensitive stage and a piston that applies incremental force to the sample on the stage. By measuring vertical tissue displacement, and incrementally applied compressive force on the tissue surface, the elastometer reports stress (force per unit area) and strain (resulting compression) of the tissue sample. The stress–strain relationship is used to derive the elastic modulus, known as the Young's Modulus (YM), which is an expression of hardness or stiffness of a material. The derived YM of the tissue sample was based on the stress–strain relation, estimated as the slope of the relation by linear curve fitting (first-order polynomial fit) in a linear range of 7.5%–15% in this study.[3] Given variability in rat size, weight and girth, and distal (affected) colon tissue, YM measurements were normalized to the YM of the intra-animal proximal colon, yielding a YM ratio.

References

1. Lubinski MA, Emelianov SY, O'Donnell M. Speckle tracking methods for ultrasonic elasticity imaging using short-time correlation. IEEE Trans Ultrason Ferroelectr Freq Control 1999;46:82–96.
2. Lubinski MA, Emelianov SY, O'Donnell M. Adaptive strain estimation using retrospective processing. IEEE Trans Ultrason Ferroelectr Freq Control 1999;46:97–107.
3. Egorov V, Tsyuryupa S, Kanilo S, et al. Soft tissue elastometer. Medical Engineering & Physics 2008;30:206–212.