Magnetic Resonance Imaging for Detection of Early Intestinal Injury of Neonatal Necrotizing Enterocolitis in a Rodent Model

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Purpose: Neonatal necrotizing enterocolitis (NEC) is a poorly understood, life-threatening illness afflicting premature infants. Research is hampered by the absence of a suitable method to monitor disease progression non-invasively. The primary goal of this research was to develop and test *in vivo* MRI methods as a tool for non-invasive early detection and staging of inflammation in the ileum of an infant rat model of NEC. In addition, MRI was used to improve understanding of necrotizing enterocolitis, specifically the progression of disease and response to therapy. We hypothesized that non-invasive MRI methods could be used for early detection of intestinal injury prior to clinical symptoms in a physiologic rat pup model of NEC.

Methods: Neonatal rats from time-dated pregnant Sprague-Dawley dams were delivered by Cesarean section at E20 following isoflurane anesthesia. Pups were fed with Esbilac puppy formula every 3 hours via an orogastric feeding catheter and stressed under 5% O_2 + 95% N_2 for 10 minutes after feeding three times a day to induce NEC. Naturally born and dam-fed neonatal rats were used as healthy controls. *In vivo* MRI studies were performed using a Bruker 9.4 Tesla scanner using a 35 mm quad coil. Rat pups were anesthetized with 0.5-1% isoflurane mixed with medical air to maintain a surgical plane of anesthesia. High-resolution anatomical MR images in coronal and axial orientations were acquired using both FLASH gradient echo sequences and RARE spin echo sequences. The in-plane resolution was ~67 microns with field-of-view of 20 mm and slice thickness of 0.25 mm. Pixel-by-pixel T_2 maps were produced using a multi-slice-multi-echo sequence. Diffusion weighted imaging (DWI) was used to obtain the apparent diffusion constant (ADC) of water and ADC maps. Pups were sacrificed at the end of MRI experiment on day 2 or 4 for histology and measurement of intestinal inflammatory cytokines. H&E stained intestinal sections were assessed for ileal damage by a pathologist to evaluate the degree of intestinal injury, from mild (1, slight separation of submucosa) to severe (3, loss of villi and necrosis) NEC.

Results: In panel **A** of Figure 1 we compare MR images as well as quantitative, pixel-by pixel T_2 and ADC maps from a control (left) and a NEC pup (right), as labeled. The pixel size in this case is 156 µm x 156 µm. Brighter ileal sections corresponding to the inflamed ileum regions are seen only in the MR image (top-right in panel A) of the NEC pup, while in the control pup no inflammation is detected in the MR image (top-left in panel A). In both panels, ileal regions, immediately proximal to cecum, are delineated by black lines. For the NEC pup, the inscribed areas show more yellow and red, indicating the ileal regions of a symptomatic rat pup with NEC have higher T_2 and ADC values than the control rat pup. An area of ~0.005 cm^2 region-of-interest in the ileal section was selected in each case for measuring T_2 and ADC values. For the mother fed, non-stressed control rat pup, average T_2 was 66.94 ± 5.58 msec and average ADC was 1.09 ± 0.04 x10^{-3} mm^2/s. For the NEC rat pup, the average T_2 was 99.41 ± 8.75 msec and average ADC was 2.08 ± 0.12 x 10^{-3} mm^2/s with p<0.003. Panel **B** shows that in pups with higher inflammatory cytokines, T_2's, ADC's, and NEC scores are higher. For one pup, as highlighted, MRI indicated inflammation correlated with elevated cytokines even when NEC score was "0" suggesting that MRI can identify inflammation prior to histologic change. Cytokine levels in NEC and disease-free groups, identified based on both H&E and MRI, were significantly different by one-way ANOVA with Bonferroni correction (p < 0.05), as depicted by (*). This suggests that MRI can be used to identify pups with elevated inflammatory activity. In panel **C**, T_2 and ADC values, as determined by MRI, are plotted as a function of NEC score, as obtained from histo-pathology.
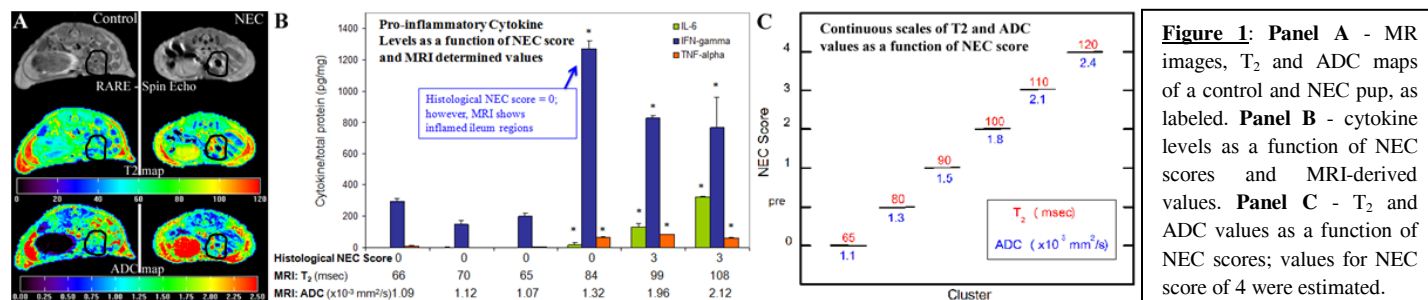

Figure 1: **Panel A** - MR images, T_2 and ADC maps of a control and NEC pup, as labeled. **Panel B** - cytokine levels as a function of NEC scores and MRI-derived values. **Panel C** - T_2 and ADC values as a function of NEC scores; values for NEC score of 4 were estimated.

Conclusion: Using high-resolution anatomical and diffusion weighted imaging, in conjunction with histological images of the excised ileal tissue samples and levels of intestinal inflammatory cytokines, for the first time we have non-invasively identified NEC in 2-4 days old rat pups. In addition, we non-invasively assessed intestinal injury prior to clinical symptoms in a physiologic rat pup model of NEC.