Akt1 deficient mice show resistance to DSS-colitis induced leak of albumin-based contrast media from the colon vasculature

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Introduction: The etiology of inflammatory bowel disease (IBD), a group of inflammatory conditions of the intestinal tract, remains largely unknown. Hallmark pathological IBD features include leukocyte infiltration, intestinal mucosal damage and ulceration. In addition, IBD is also associated with major alterations and dysfunction of the intestinal microvasculature, as indicated by clinical and preclinical studies that delineated a central role for microcirculation in initiation and perpetuation of inflammatory processes (Hulten et al. Gastroenterology 1977, 72:388-396; Deban et al., Am J Pathol 2008, 172:1457-1466). Colitis induced by dextran sodium sulfate (DSS, a detergent introducing lesions in the intestinal epithelium), is the most widely used chemically induced model of ulcerative colitis. Adding DSS to the drinking water for several days induces an acute intestinal inflammation with bloody diarrhea and ulcerations (Wirtz et al., Nat Protoc 2007, 2:541-546). Akt1, a protein kinase, is a major mediator of angiogenic signaling, acting downstream of vascular endothelial growth factor (VEGF). Akt1, also known as PKBalpha, is an intracellular kinase that phosphorylates target substrates to regulate crucial aspects of growth and survival in endothelial cells. Imaging of the colon using macromolecular delayed contrast enhanced (DCE)-MRI can be used to quantify the permeability surface area product (PS), which provides a measure of blood vessel permeability. The purpose of this study was to elucidate the role of Akt1 in DSS colitis of the colon using *in vivo* MRI of Akt1 deficient mice.

Methods: Six wild type (WT, $Akt^{+/+}$), 2 Akt1 heterozygote ($Akt^{+/-}$), and 3 Akt1 knockout ($Akt^{-/-}$) mice on a C57B6 background were exposed to dextran sulfate sodium (DSS, an irritant to the colon) in the drinking water for 7 days. Afterwards mice were imaged on a 9.4T BioSpec MRI system using a quadrature resonator for excitation and detection (Bruker, Germany). Biotin-BSA-GdDTPA, a macromolecular contrast agent, was prepared as reported previously (Dafni et al., NMR Biomed 2002, 15:120-131) and injected intravenously (IV) as a bolus through a tail vein catheter. 3D Gradient echo (3D-GE) images of the lower abdomen were acquired before and sequentially for 30 minutes after IV injection of the contrast agent. (Imaging parameters: TR 10ms; TE 4ms; two averages; spectral width 50,000Hz; matrix 128x128x64; zero-filled to 256x256x128; FOV 40x40x40mm). A series of variable flip angle (5, 15, 30, 50 70) T1-weighted 3D-GE images were acquired prior to contrast administration in order to determine the endogenous R1. Pixel-by-pixel analysis was done on a stand alone workstation using MATLAB (Mathworks, USA) to generate the concentration of biotin-BSA-GdDTPA in an ROI that contained the colon for selected slices (Aychek and Vandoorne et al., MRM, submitted). After averaging the concentration of contrast material in the selected ROI (the whole colon) by the concentration in the vena cava, PS values were calculated from a linear regression over data from the first 15min. MRI data were validated in histological sections by leakage of fluorescently labeled BSA-FAM injected together with the MRI contrast agent.

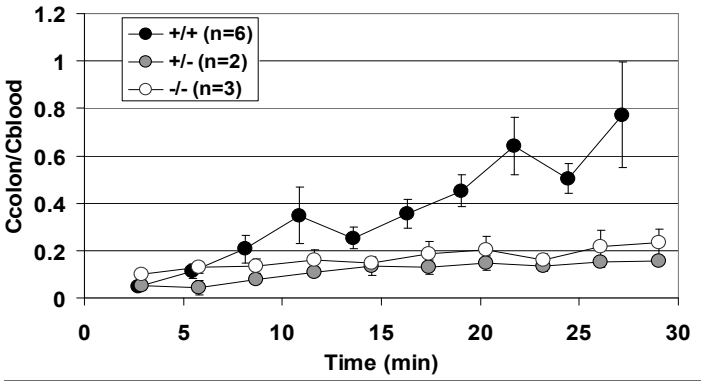

Fig. 1. Time-course of the ratio of contrast agent concentration in blood and colon using *in vivo* dynamic DCE-MRI for 30 minutes post contrast (10 time points). For wild type (+/+), Akt1 heterozygote (+/-), and Akt1 null mice (-/-).

Results: Time-course measurements of biotin-BSA-GdDTPA distribution for $Akt^{+/+}$, $Akt^{+/-}$, and $Akt^{-/-}$ mice are shown in Figure 1. $Akt^{+/+}$ mice demonstrated a rapid increase in the ratio of colon:blood contrast agent concentration. In contrast, both Akt1 heterozygote and knockout deficient mice demonstrated an attenuated increase in leakage of contrast agent into the colon vasculature. Measurements of PS in all mice are shown in Figure 2. As expected, $Akt1^{-/-}$ mice demonstrated significantly decreased PS compared to $Akt^{+/+}$ mice. Preliminary results in Akt1 heterozygous mice also indicate a decreased permeability of albumin-based contrast agent in the colon, implying less leakage of plasma proteins and thus, limited disease progression. Analysis of histological sections confirmed the decreased leakage of fluorescently labeled BSA-FAM in $Akt^{+/-}$ and $Akt^{-/-}$ mice compared to $Akt^{+/+}$ mice. Despite the reduced permeability, histological examination confirmed the presence of colitis in the form of ulcers and wall thickness independent of Akt1 expression.

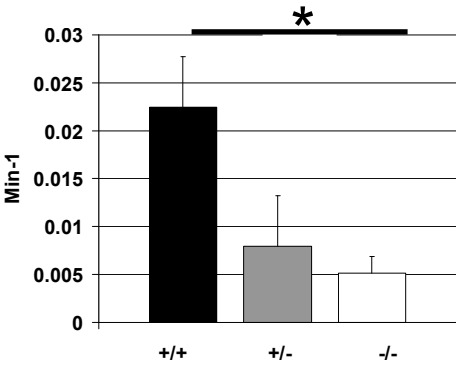

Fig. 2. Permeability surface area product (PS; the slope of concentration curve normalized to blood concentration) for wild type, Akt heterozygote, and Akt null mice. (Student T test, One-way ANOVA *P<0.01; Mean ± stderror).

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