HYALURONAN AS POTENTIAL MODULATOR OF THE GUT NEURO-IMMUNE FUNCTION AFTER INTESTINAL ISCHEMIA/REPERFUSION INJURY

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BACKGROUND: Hyaluronan (HA), an extracellular matrix glycosaminoglycan component, appears to be involved in the pathogenesis of chronic inflammatory bowel disease (IBD) by modulating toll-like receptor (TLR) pathways. Recently, HA was shown to maintain enteric neuron homeostasis by forming a well-structured perineuronal net, which is altered during inflammation. Since IBD often includes episodes of ischemia, in this study we aimed to assess the role of HA in the morphology and activity of the rat small intestine neuromuscular compartment after ischemia reperfusion (I/R).
MATERIALS AND METHODS: In vivo I/R injury was induced by clamping the superior mesenteric artery for 60 min, followed by 24 hours of reperfusion in adult male Wistar rats (300-350g), after general anesthesia. In some experiments, the HA synthesis inhibitor, 4-methylumbelliferone (4-MU, 25mg/kg), was intraperitoneally administered to normal (CTR), sham-operated (SH) and I/R animals 24 h before euthanasia.
RESULTS: In the I/R group, treatment with 4-MU dampened the marked increase of HA levels and density index of fluorescent HA binding protein (HABP) staining as well as the higher mRNA levels of the functional HA synthase, HAS2, in the small intestine neuromuscular and submucosal compartments. The increased number of neutrophils infiltrating the muscularis propria, myenteric ganglia and submucosal layer in the I/R group was significantly reduced by 4-MU treatment. In longitudinal muscle myenteric plexus (LMMP) preparations and in the submucosal layer of I/R rats TLR2 mRNA levels significantly increased and were reduced by 4-MU treatment. In LMMP but not in submucosal preparations TLR4 mRNA levels increased and were reduced by 4MU. The efficiency of the GI transit, measured as geometric center of non-absorbable FITC-dextran, was significantly reduced in the I/R group and was further reduced by 4-MU. In the I/R group, carbachol- and electrical field- (EFS, 0.1-40 Hz) stimulated contractions and EFS-induced (10 Hz) non-cholinergic non-adrenergic (NANC) relaxations were reduced with respect to both CTR and SH groups. I/R-mediated inhibition of EFS contractions, but neither of CCh-induced contractions nor of NANC relaxations, was abolished by 4-MU treatment.
CONCLUSIONS: Our data suggest that I/R injury increases HA levels in the neuromuscular and submucosal compartments of the rat small intestine which may depend on HAS2 transcription modulation and involves HA-mediated changes in TLR2 and TLR4 expression. During I/R, HA influences the GI transit mainly by regulating excitatory enteric pathways. Changes in gut HA homeostasis may have a role in development of enteric motor dysfunction after I/R.