HYALURONAN INVOLVEMENT IN NEUROMUSCULAR ADAPTATION TO INTESTINAL ISCHEMIA/ REPERFUSION INJURY

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Introduction: Intestinal ischemia/reperfusion (I/R) injury, caused by an insufficient supply of blood flow to all or part of the gastrointestinal (GI) tract, has severe consequences on the different cell types constituting the enteric microenvironment including enterocytes, smooth muscle cells, enteric glial cells and neurons. Myenteric neurons are especially sensitive, and can be irreversibly damaged. From a functional viewpoint, I/R-induced neuronal loss entails hindered food digestion and slowing of transit. Myenteric neurons synthesize hyaluronan (HA) to form a well-structured perineuronal net, which undergoes derangement when myenteric ganglia homeostasis is perturbed, i.e. during inflammation. Since chronic inflammatory bowel diseases often include episodes of ischemia, in this study we aim to evaluate possible changes in HA homeostasis in myenteric ganglia after an *in vivo*-induced I/R damage in rats.

Materials and methods: In vivo ischemia reperfusion (I/R) injury was induced by clamping the superior mesenteric artery for 60 min, followed by 24hours of reperfusion in adult male Han 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 for 24h.

Results: In longitudinal muscle myenteric plexus whole-mount preparations, the density index of fluorescent HA binding protein (HABP) staining was significantly higher in the I/R group, and was reduced after 4-MU treatment. Accordingly, ELISA quantification of HA in LMMPs revealed a significantly increased level of the glycosaminoglycan in the I/R group with respect to CTR and SH groups, which was reduced by 4-MU treatment. HA synthase 1and 2(HAS1and HAS2) mRNA levels increased in the I/R group with respect to CTR, while HAS1and HAS2protein levels were unchanged with respect to CTR. The efficiency of the GI transit, measured as geometric center of non-absorbable FITC-dextran, was significantly reduced in I/R groups vs CTR and SH groups, and was further reduced after 4-MU treatment. CCh and electrical field (EFS, 0.1-40 Hz) stimulated contractions and EFS-induced (10 Hz) NANC relaxations, in the presence of 1μM guanethidine + 1μM atropine, were reduced in the I/R group with respect to both CTR and SH groups. I/R-induced decrease of both EFS contractions and NANC relaxations, but not of CCh-induced contractions, was abolished after 4-MU treatment.

Discussion and conclusions: Overall, our data suggest that, in the neuromuscular compartment of the rat small intestine, I/R injury increases HA levels, which do not depend on I/R-mediated regulation of HAS1and HAS2protein expression. Changes in HA homeostasis may influence GI transit, by modulating both excitatory and inhibitory intestinal motor pathways after an ischemic insult. We cannot exclude that modulation of HA synthesis in these conditions may ameliorate derangement of the enteric motor function preventing, at least in part, the development of dysmotility.