HOMEOBOX TRANSCRIPTION FACTORS OTX1 AND OTX2 INVOLVEMENT IN RAT MYENTERIC PLEXUS PLASTICITY AFTER DNBS-INDUCED COLITIS

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Introduction: Inflammatory bowel diseases (IBD) are associated with profound alterations of intrinsic myenteric plexus circuitries, occurring also at intestinal sites distant from the injury. Such alterations may underpin development of disturbed motor function, which represents one of the main symptoms of these gut disorders. The mechanism/s underlying such derangement, however, have not been completely clarified yet. In this study we investigated the possible involvement of homeobox gene pathways, OTX1 and OTX2 in the rat distal colon and small intestine myenteric plexus of control animals and after 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis. OTX1 and OTX2 are nuclear transcription factors participating to adaptation during inflammation and underlying tumor growth in both the CNS and in the periphery, including the enteric nervous system (ENS).

Materials and methods: Experimental colitis was induced in male Sprague-Dawley rats (weight 250-300g) by administration of a single dose (30 mg) of DNBS. Controls (CTR) were given ethanol 50% (vehicle). Animals were euthanized 6 days after the induction of colitis, when the intestinal inflammatory process is maximal. Distribution of OTX1 and OTX2 was immunohistochemically evaluated in longitudinal muscle myenteric plexus (LMMP) whole-mount preparations and in paraffin sections. OTX1 and OTX2 mRNAs and protein levels were evaluated by qRT-PCR and Western blotting. All data are expressed as mean±SEM and statistical significance was calculated with one-way ANOVA with Tukey's post hoc test.

Results: In small intestine and colonic LMMPs, myeloperoxidase activity, TNFa, IL1b, IL6, VEGFα and HIF1α mRNA levels, evaluated as indicators of the inflammatory damage, significantly increased after DNBS treatment. DNBS-treatment induced profound morphological and histological alterations mainly in the distal colon, while the number of myenteric neurons was significantly reduced in both regions. The number of OTX1 immunopositive neurons/area was significantly higher both in small intestine (10.09±1.20% n=4) and colon (54.32±6.52% n=6) preparations obtained from DNBS-treated animals with respect to CTR (5.89±1.20%, n=4; 5.74±2.5%, n=6, respectively). OTX1 specific antibody prevalently labeled enteric glial cells, as evidenced by co-staining with the glial marker S100b. OTX1 immunostaining was highly superimposable with the inducible nitric oxide synthase (iNOS) in both regions. The number of OTX2-immunopositive neurons/area was significantly higher both in small intestine (42.24±2.8% n=8) and colon (36.78±4.51% n=6) preparations obtained from DNBS-treated animals with respect to CTR (7.32±1.16%, n=8; 10.23±2.5%, n=6, respectively). OTX2 specific antibody labeled only myenteric neurons and was highly co-localized with neuronal nitric oxide synthase (nNOS). OTX1 and OTX2 mRNA and protein levels significantly increased both in small intestine and colonic preparations obtained from DNBS-treated animals.

Discussion and conclusion: Our data provide evidence that DNBS treatment in rats increases inflammatory markers not only in the site of inflammation, but also distally, in the small intestine. In addition, we demonstrate that inflammation alters OTX1 and OTX2 levels in the gut neuromuscular compartment, possibly contributing to derangement of myenteric ganglia. We cannot exclude that modulation of OTX1 and OTX2 expression may rescue myenteric neurons thus contributing to ameliorate motor function alterations associated with inflammation.