CHANGES IN THE EXPRESSION OF HOMEBOX TRANSCRIPTION FACTORS OTX1 AND OTX2 IN THE RAT MYENTERIC PLEXUS AFTER DNBS-INDUCED COLITIS

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Background and Aim: a complex interplay of immunological, genetic and environmental factors are involved in the pathogenesis of inflammatory bowel diseases (IBD), whose symptoms include disturbed motor gastrointestinal (GI) functions. These latter may depend upon profound alterations of intrinsic myenteric plexus circuitries (1). The mechanism/s underlying such derangement, however, have not been completely unraveled yet. In this study we investigated the possible involvement of homeobox gene pathways, OTX1 and OTX2 in the rat colon myenteric plexus of control animals and after 2,4-dinitrobenzene sulfonic acid (DNBS)-induced colitis. OTX1 and OTX2 represent nuclear transcription factors involved in neurodegeneration, as recently demonstrated also in the enteric nervous system (ENS) (2).

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. Expression of OTX1 and OTX2 mRNAs and protein was assessed in longitudinal muscle myenteric plexus (LMMP) preparations by means of qRT-PCR and western blotting. Immunohistochemical investigations were carried out on LMMP whole mount preparations to localize OTX1 and OTX2. All data are expressed as mean±SEM and statistical significance was calculated with Student’s t test for unpaired data.

Results: in colonic LMMPs, myeloperoxidase activity, VEGF and HIF1α mRNA levels, evaluated as indicators of the inflammatory damage, significantly increased after DNBS treatment. The number of OTX immunopositive neurons was significantly higher 23.0±3.0% (n=3) in preparations obtained from DNBS-treated animals than in CTR 9.3±2.0% (n=3). OTX1 specific antibody prevalently labeled enteric glial cells, while OTX2 specific antibody labeled myenteric neurons. OTX1 and OTX2 mRNA levels significantly increased (P<0.001) in preparations obtained from DNBS-treated animals. OTX1 protein levels significantly increased (P<0.05) after DNBS-treatment.

Conclusion: our data indicate for the first time that inflammation alters OTX1 and OTX2 levels in the gut neuromuscular compartment, possibly contributing to derangement of myenteric ganglia. Overall, these data suggest that homeobox transcription factors may play an important role in the regulation of the adult ENS, contributing to the pathogenesis of important GI disorders.

References

1. Lomax et al., Neurogastroenterol Motil 2005; (17): 4-15
2. Filpa et al., Am J Physiol Gastrointest and Liver Physiol 2017; (312): G374-G389