Enteric bacteria alterations and inflammation associated with diclofenac-induced enteropathy: preventive effects of rifaximin

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Non steroidal anti-inflammatory drugs (NSAIDs), besides exerting detrimental effects on the upper digestive tract, can also damage the small and large intestine. Although the underlying mechanisms remain unclear, there is evidence that enteric bacteria could play a prominent role. In particular, NSAIDs increase mucosal permeability, thus facilitating the adhesion and penetration of bacteria, which trigger the inflammatory cascade *via* activation of toll-like receptors (TLRs). Based on this background, the present study examined the effects of rifaximin, a poorly absorbed antibiotic, on enteric bacterial load and composition as well as small bowel inflammatory responses in a rat model of diclofenac-induced enteropathy. Enteropathy was induced in male rats (40-weeks old) by intragastric diclofenac administration (4 mg/kg BID) for 14 days. Control animals received drug vehicle (0.3 ml of 1% methylcellulose). A group of rats received rifaximin polymorph-alpha as an extended intestinal release (EIR) formulation, consisting of coated microgranules (rifaximin-EIR, 50 mg/kg BID), 1 hour before diclofenac (n=6-7 per group). At the end of treatments, feces were collected to quantify calprotectin content by ELISA. Ileum was excised and processed for the evaluation of: 1) tissue myeloperoxidase levels (as an index of neutrophil infiltration); 2) bacterial total load and quantitative analysis of strains, *via* 16S real-time PCR; 3) expression of TLR-2/4 and activation of downstream signaling as phosphorylated nuclear factor kB subunit p65 (NF-kB p65) and myeloid-differentiation primary response-gene 88 (MyD88), by Western blot. In control animals, myeloperoxidase and calprotectin levels were 6.0±1.1 ng/mg and 2.5±0.2 ng/mg, respectively. These parameters were significantly increased in diclofenac-treated rats (myeloperoxidase: +290%; calprotectin: +52%). Ileal specimens from control animals were found to contain a total bacterial load of $4.66\pm1.01\times10^{10}$; the *Bacterioidetes* phylum was $0.14\pm0.05\times10^{10}$ and the *Firmicutes* phylum was $0.26\pm0.03\times10^{10}$. All these bacterial values increased after treatment with diclofenac. The expression of TLR-2/-4, NF-kBp65 and MyD88 in diclofenac-treated animals was higher, as compared with control animals (respectively: +104%, +23%, +147%, +71%). In rats treated with diclofenac plus rifaximin-EIR, myeloperoxidase and calprotectin levels were lower as compared with diclofenac alone (respectively: -74% and 89%). In this setting, the bacterial total load decreased by -88%, with a significant reduction of *Bacteroidetes* and *Firmicutes*, and the increased expression levels of TLR-2/-4, NF-kBp65 and MyD88 returned towards control values. In small bowel, treatment with diclofenac leads to quantitative and qualitative alterations of enteric bacteria that are associated with an increased expression/activation of TLR-2/-4 and consequent tissue inflammation. Under these conditions, rifaximin-EIR counteracts the bacterial changes and promotes a normalization of related inflammatory responses. These peculiar pharmacological actions of rifaximin may represent the underlying mechanism(s) of its preventive activity against NSAID-induced intestinal damage, recently observed in rats (Fornai et al., 2015) and humans (Scarpignato et al., 2015) in our Institutions.

Fornai et al. (2015). *Gastroenterology* 148 (Suppl 1): A-398.
Scarpignato et al. (2015). *Gastroenterology* 148 (Suppl 1): A-307.