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Use of a balanced dual cyclooxygenase-1/2 and 5-lypoxygenase inhibitor in experimental colitis

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ABSTRACT

Cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) play an important role in inflammatory bowel diseases (IBDs). We investigated the effects of flavocoxid, a dual COX/LOX inhibitor, in experimental colitis induced with either dinitrobenzenesulfonic acid (DNBS) or dextrane sulphate sodium (DSS)

In the first model, colitis was induced in rats by a single intra-colonic instillation (25 mg in 0.8 ml 50% ethanol) of DNBS; after 24 h animals were randomized to receive orally twice a day, flavocoxid (10 mg/kg), zileuton (50 mg/kg), or celecoxib (5 mg/kg). Sham animals received 0.8 ml of saline by a single intra-colonic instillation. Rats were killed 4 days after induction and samples were collected for analysis. In the second model, colitis was induced in rats by the administration of 8% DSS dissolved in drinking water; after 24 h animals were randomized to the same above reported treatments. Sham animals received standard drinking water. Rats were killed 5 days after induction and samples were collected for analysis.

Flavocoxid, zileuton and celecoxib improved weight loss, reduced colonic myeloperoxydase activity, macroscopic and microscopic damage, and TNF-α serum levels. Flavocoxid and celecoxib also reduced malondialdheyde, 6-keto $PGF_{1\alpha}$ and PGE-2 levels while flavocoxid and zileuton decreased LTB-4 levels. In addition, flavocoxid treatment improved histological features and apoptosis as compared to zileuton and celecoxib; moreover only flavocoxid reduced TXB2, thus avoiding an imbalance in eicosanoids production.

Our results show that flavocoxid has protective effect in IBDs and may represents a future safe treatment for inflammatory bowel diseases.

1. Introduction

The pathogenesis of inflammatory bowel diseases (IBDs) is still poorly understood, however, increasing evidence sustains the interaction between environmental, genetic, and immunological factors (Podolsky, 2002). During IBD reactive metabolites of oxygen (ROS) stimulate: the release of chemokines; activation of lipid peroxidation; recruitment of neutrophils; the release of TNF-α (Pavlick et al., 2002; Rahimian et al., 2010); the activation of NF-κB and the apoptotic kinase JNK (Shaulian and Karin, 2012). Of the pro-inflammatory genes that are induced by ROS, it is noteworthy the expression of cyclooxygenase (COX-2) and 5-lipoxygenase (5-LOX). In IBDs COX-2 mediates the increase in PGE2, important for the development and maintenance of an inflammatory microenvironment (Sklyarov et al., 2011). Concomitantly, an increased activity of 5-lipooxygenase (5-LOX) lead to an enhanced formation of leukotriene A4 (LTA-4), further transformed by the LTA-4 hydrolase into LTB-4, especially in macrophages and polymorphonuclear leukocytes. LTB-4 attracts leukocytes to the site of inflammation, promoting their adhesion to the inflammed and damaged tissue (Singh et al., 2004), thus amplifying the inflammatory cascade in IBDs (Holma et al., 2007). Aspirin and NSAIDs are known to suppress COX expression/activity and eicosanoid production, however, inhibition of COXs may lead to cardiotoxicity due to an imbalanced production of pro-thrombotic eicosanoids (e.g. increased TxA2) and anti-thrombotic eicosanoids (e.g. decreased PGI2) (Bresalier et al., 2005; Solomon et al., 2005). Since the inhibition of one or both COX enzymes may shunt arachidonic acid metabolism to the 5-LOX pathway, zileuton, an

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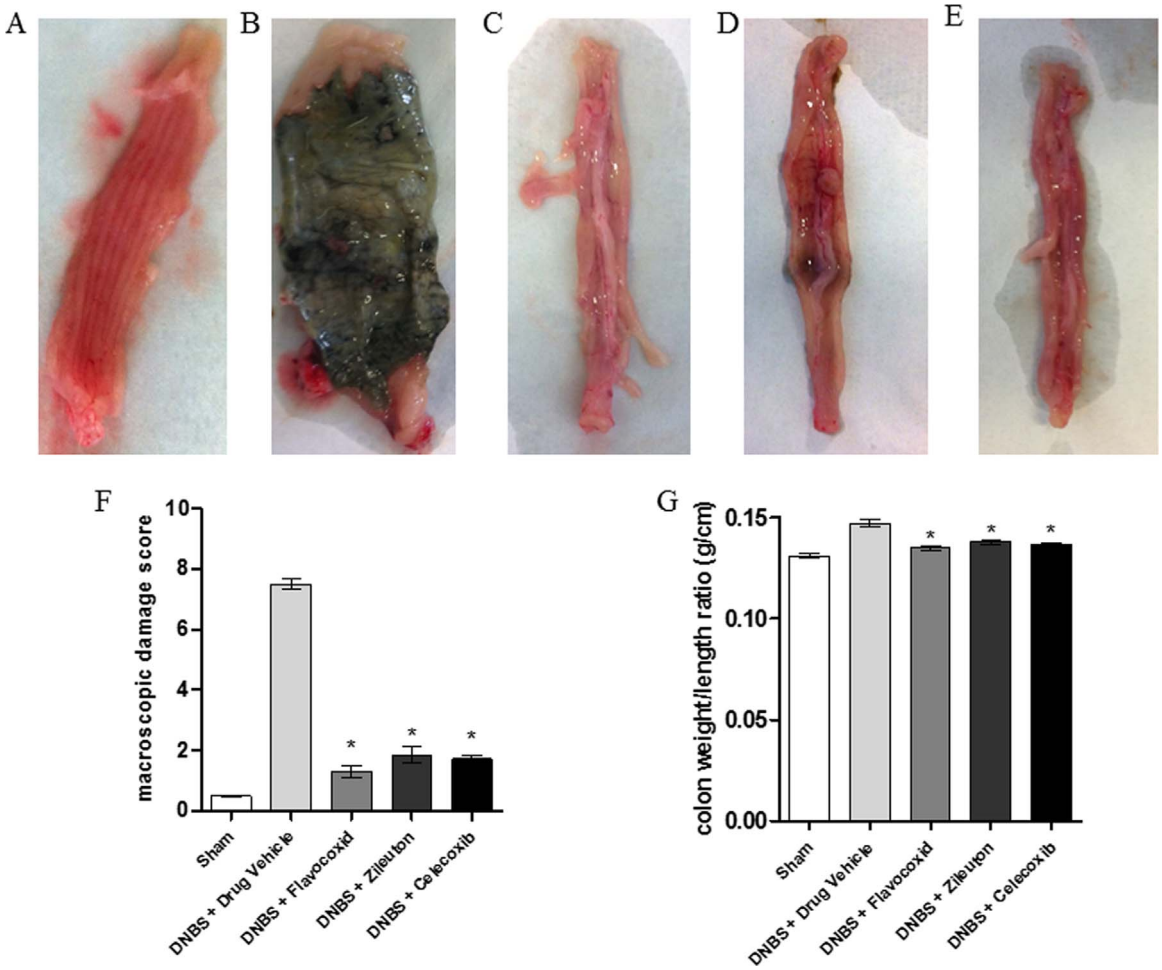

Fig. 1. Colon tissues from sham (A), DNBS + drug vehicle (B), DNBS+Flavocoxid (C), DNBS+Zileuton (D), DNBS+Celecoxib (E) together with the graph representing macroscopic damage scores (F) and colon weight/length ratio (G). Values are expressed as the mean and S.E.M. *P < 0.0001 vs DNBS+drug vehicle group. n=7 for each group.

active 5-lipoxygenase inhibitor, was compared in a trial versus mesalazine and placebo. However, zileuton was not superior to placebo in maintaining remission of symptoms in ulcerative colitis (Hawkey et al., 1997). Thus, it appears that a targeted inhibition of either COX or 5-LOX does not represent the proper therapeutic strategy to block the inflammatory response in IBDs. It can also be speculated that a balanced inhibition of both enzymes might have synergistic effects and could be the key point for an effective treatment of IBDs. Some natural compounds, termed medical foods, demonstrated their efficacy and safety in the dietary management of specific diseases. A well characterized medical food, flavocoxid is a proprietary blend of baicalin and catechin, concentrated and standardized to greater than 90% purity. Flavocoxid, has been also demonstrated to be a balanced dual inhibitor of the peroxidise moieties of COX and 5-LOX, blunting the proinflammatory response (Burnett et al., 2011), as demonstrated in LPS-stimulated macrophages (Altavilla et al., 2009), acute pancreatitis (Polito et al., 2010), cecal ligation and puncture (Bitto et al., 2012). In addition to COX and 5-LOX inhibition flavocoxid may reduce reactive oxygen species including hydroxyl radical, superoxide anion radical, and hydrogen peroxide, modulating in turn, NF-κB and TNF-α production (Bitto et al., 2014). To date, a dual inhibitor of both COX and 5-LOX pathways has not been investigated in experimental colitis. Therefore, we aimed at evaluating the potential therapeutic effect of Flavocoxid, in experimental colitis induced with either DNBS or DSS, and to compare its effect to the 5-LOX inhibitor Zileuton and to COX-2 inhibitor, celecoxib.

2. Materials and methods

2.1. Animal and treatments

All animal procedures were in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki), the ARRIVE Guidelines (McGrath et al., 2010), and authorised by the Animal Ethics Committee of the Department Clinical and Experimental Medicine (approval #04/14), and all efforts were made to minimize suffering.

A total of 70 male Sprague–Dawley rats (250–300 g) were purchased from Charles River Laboratories (Calco, Milan, Italy). Animals were maintained in plastic cages under standard environmental conditions with water and food ad libitum. After 1 week of acclimation to the laboratory environment, animals were randomly assigned to 10 groups of 7 animals each.

In preliminary experiments, flavocoxid dose was titrated against the effects on PGE-2 expression in 30 animals. The day after DNBS administration 6 animals/group received flavocoxid twice a day at 5, 10, 20, and 40 mg/kg (by gavage) and the treatments lasted 4 days. This experiment identified 20 mg/kg/day as the optimal dose to be used in the further experiments (Supplemental Figure 1). Zileuton and celecoxib dose and route of administration were chosen according to previously published