In rat fibrotic colon TGF-beta/SMAD signalling is modulated by cyclooxygenases inihibitors

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Aims. Fibrotic remodelling of gut wall can occur as a consequence of chronic inflammation, and can have serious clinical consequences. This process is characterized by an excessive deposition of collagen and a rearrangement of other extracellular matrix components. Cyclooxygenase isoforms (COX-1, COX-2) have been implicated in the development of fibrosis at gastrointestinal sites. Under bowel inflammation, transforming growth factor beta (TGF-beta) has been identified as the main regulator of fibrotic remodelling. The present study investigated the effects of cyclooxygenase inhibitors on pro-fibrotic signalling mediated by the TGF-beta/SMAD pathway in experimental colitis.
Methods. Colitis was induced in rats by intrarectal 2,4-dinitrobenzenesulfonic acid (DNBS, 30 mg/rat in 0.25 ml ethanol 50%). After 6 days, systemic [body and spleen weight] and tissue inflammatory parameters [macroscopic and microscopic damage] were assessed. Three days before colitis assessment, the animals were treated daily with indomethacin (IND, non-selective COX-1/COX-2 inhibitor, 2 mg/kg), SC-560 (SC, selective COX-1 inhibitor, 2.5 mg/kg), or celecoxib (CEL, selective COX-2 inhibitor, 1 mg/kg) by intragastric gavage. At the time of sacrifice, functional and molecular tests were carried out. Peristaltic activity of control and inflamed colonic segments was studied in vitro by a modified Trendelenburg set-up. COX-1, COX-2, collagen I and III, fibronectin, matrix metalloproteinase(MMP)-2 and MMP-9 protein expression were analyzed by western blot assays. COX-2 was also analyzed by immunohistochemistry. Collagen fibers (Van Gieson) and elastic fibers (orcein) were examined by histochemistry. The expression of molecular factors involved in TGF-beta signalling (TGF-beta, RhoA and SMAD6, as well as phosphorylated SMAD2, p38, ERKs and JNK), and cellular proliferation and apoptotis (PCNA, Akt and caspase-3) were analyzed by western blot.
Results. Animalswith colitis displayed an impairment of colonic peristalsis, as shown by a significant decrease in compliance and maximal ejection pressure, as well as an increase in the threshold pressure and luminal volume required to trigger peristalsis. Moreover, histochemistry showed an increased deposition of collagen fibers in parallel with a dramatic decrease in elastic fibers. IND, SC and CEL inhibited collagen deposition, and reverted the loss of elastic fibers. Based on western blot, IND, SC and CEL counteracted the increased expression of collagen III (from 7.4 to 3.9, 4.8, and 5.1 folds, respectively), fibronectin (from 10 to 3.0, 3.8 and 3.6 folds, respectively) and, to a lesser extent, collagen I. In the inflamed/fibrotic colon, western blot analysis revealed an increased expression of TGF-beta, PCNA, caspase-3, pAkt, p-p38, RhoA and pSMAD2. By contrast, the expression of SMAD6, pERKs and pJNK was reduced. In this setting, IND, SC and CEL counteracted the increased expression of TGF-beta, RhoA, pSMAD2 and p-p38, and induced the expression of SMAD6.
Conclusions. In the DNBS model of colitis, bowel fibrosis is associated with impaired colonic peristalsis and is characterized by an enhanced collagen/fibronectin deposition in parallel with an elastic fiber reduction. The pharmacological blockade of COX-1 and COX-2 is able to counteract the fibrotic remodelling of inflamed colon, and this action appears to depend on the modulation of TGF-beta-dependent SMAD signalling, particularly through an induction of the inhibitory protein SMAD6 and a reduction of the phosphorylated status of SMAD2.