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The vitamin D analog TX527 ameliorates disease symptoms in a chemically induced model of inflammatory bowel disease

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ABSTRACT

The vitamin D system plays a critical role in inflammatory bowel disease as evidenced by the finding that both vitamin D deficient mice and vitamin D receptor knockout mice are extremely sensitive to dextran sodium sulfate (DSS)-induced colitis. Moreover, the active form of vitamin D, 1α,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] is an important immunomodulator that ameliorates the pathogenesis of inflammatory bowel disease. However, therapeutic application of 1,25$(OH)_2D_3$ is hampered by its calcemic activity. Previous work illustrated that the analog 1α,25$(OH)_2$-19-nor-14,20-bisepi-23-yne-vitamin D_3 (TX527) has potent antiproliferative effects with limited calcemic activity. In the present study we demonstrated that TX527 ameliorated disease symptoms in a DSS-induced model of inflammatory bowel disease. TX527 significantly attenuated disease scores, by suppressing bleeding and diarrhea. Colon length was significantly elevated at the end of the experiment. Histological examination indicated that TX527 diminished mucosal damage and crypt loss and suppressed the infiltration of immune cells in DSS-induced colitis mice. Furthermore, transcript levels of inflammatory cytokines such as IL-1, IL-6, IFN-γ and TNF-α were significantly down-regulated in colonic mucosa of mice with colitis. Moreover, transcript levels of the gastrointestinal glutathione peroxidase 2, which acts as a radical scavenger, were significantly down-regulated after TX527 treatment in DSS-colitis mice. These results indicate that TX527 may have a therapeutic value in the setting of inflammatory bowel disease.

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Contents

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1. Introduction

Inflammatory bowel diseases (IBD) are chronic, relapsing gastrointestinal disorders characterized by chronic inflammation of the intestine, and are caused by multiple factors, among which genetic and environmental factors [1]. The pathogenesis of IBD remains unclear, but imbalances between pro-inflammatory mediators, including reactive oxygen mediators, cytokines, and neutrophil infiltration on the one hand and anti-inflammatory mechanisms on the other hand are believed to play a central role in modulating inflammation [1,2]. Pro-inflammatory cytokines that are involved in the induction and perpetuation of intestinal inflammation in ulcerative colitis (UC) include tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-1 (IL-1), IL-6 and IL-12 and control of these cytokines was shown to be beneficial in spontaneous and induced colitis models [3].

Several studies suggested vitamin D as an environmental factor that contributes to IBD development. Moreover, a causal role for the vitamin D system in IBD is suggested by the fact that vitamin D deficiency as well as vitamin D receptor (VDR) deficiency exacerbate experimental IBD in the IL-10 knockout mice and in dextran sodium sulfate (DSS)-induced colitis [4,5]. The active form of vitamin D, 1α,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$] exerts its effects by binding to the VDR that acts as a ligand-activated transcription factor and modulates the expression of target genes. $1,25(OH)_2D_3$ is an important immunomodulator that ameliorates the pathogenesis of Th1-mediated auto-immune diseases including IBD [6]. However, therapeutic application of $1,25(OH)_2D_3$ is hampered by its calcemic activity. Interestingly, analogs of $1,25(OH)_2D_3$ with enhanced immunomodulatory capacities and reduced calcemic effects were shown to improve disease symptoms in experimental colitis models by increasing levels of anti-inflammatory cytokines [6]. Moreover, the analog 1α,$25(OH)_2$-19-nor-14,20-bisepi-23-yne-vitamin D_3 (TX527) was demonstrated to inhibit cell proliferation and TNF-α production in peripheral blood mononuclear cells from patients with Crohn's disease [6,7].

2. Materials and methods

2.1. Chemicals and reagents

$1,25(OH)_2D_3$ was obtained from Sigma–Aldrich (St. Louis, MO, USA). The vitamin D analog TX527 [19-nor-14,20-bisepi-23-yne-$1,25(OH)_2D_3$] was synthesized by M Vandewalle and P De Clercq (University of Ghent, Ghent, Belgium) and obtained from Théramex (Monaco, France).

2.2. Experimental animals

Eight weeks old female C57Bl/6 mice (18–20 g) (Harlan) were housed 5/cage in an animal room with 12 h dark/light cycles and constant temperature. Food and water were supplied ad libitum. All animal experiments were approved by the ethical committee of the KU Leuven.

2.3. Induction of experimental colitis

Experimental colitis was induced by administration of 2.5% DSS in drinking water. After a 1-day exposure to DSS, mice were given normal drinking water for 2 days. Thereafter, mice received drinking water with 2.5% DSS for 5 days, after which the mice were resumed on normal drinking water for the remainder of the experiment. Mice were killed 5 days after the last DSS administration. Fresh DSS solutions were prepared every other day. Mice were divided into 4 experimental groups. The first group received normal drinking water throughout the whole experiment and was treated with arachis oil ($n = 15$). The second group was given 2.5% DSS in drinking water and mice were treated with arachis oil ($n = 15$). Groups 3 and 4 received 2.5% DSS in drinking water and were treated with 0.5 μg/kg/d $1,25(OH)_2D_3$ or 3 μg/kg/d TX527, respectively (6 out of 7 days) ($n = 15$ in each group). Treatment was started 3 days before the administration of DSS. Therefore, the complete duration of the experiment was 12 days and treatment with $1,25(OH)_2D_3$ and TX527 preceded the exposure to DSS. $1,25(OH)_2D_3$ and TX527 were diluted in arachis oil and 60 μl was administered rectally. Body weight, stool consistency and gross bleeding were recorded daily. Disease activity index (DAI) was assessed as described previously [8]. At the end of the experiment, mice were euthanized by CO_2 inhalation. Colons were separated from the distal rectum and colon length was measured between the colon-cecal junction and the distal rectum.

2.4. Calcemic parameters

Serum was collected at the end of the experiment and serum calcium levels were determined by a colorimetric assay (Synchron CX4, Beckman Instruments, USA).

2.5. Histopathology

The resected large intestine was rolled up, fixed overnight in 4% fresh paraformaldehyde and embedded in paraffin. For histopathological analysis, tissue sections were stained with hematoxylin and eosin (H&E). Microscopic sections were graded by the number and severity of lesions. The degree of transmural inflammation (epithelium, submucosa, and muscularis) was calculated using a previously described scoring system [9].

2.6. Quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR)

Colonic mucosa was scraped off from the most distal cm of the colon descendens and total RNA was extracted with the High Pure RNA Isolation Kit (Roche) and used for quantitative reverse polymerase chain reaction (qRT-PCR) analysis. One microgram RNA was reverse transcribed using SuperScriptII (Invitrogen, Carlsbad, CA, USA) and the resulting cDNA (1/10 diluted) was used as a template in PCRs with the Fast SYBR Green Master Mix (Applied Biosystems Inc., Foster City, CA, USA). PCR primers were purchased from Eurogentec (Liège, Belgium) and their sequences are available upon request. The expression level of the gene of interest was normalized to the expression of β-actin.

2.7. Statistics

All statistical analyses were performed with the software program Statistica (StatSoft Inc., Tulsa, OK, USA). Results were expressed as the mean and SEM. Analysis of variance analyses (ANOVA) were followed by Fisher's least significant difference (LSD) multiple comparison test. $p < 0.05$ was accepted as significant.