The Interleukin-1β-Converting Enzyme Inhibitor Pralnacasan Reduces Dextran Sulfate Sodium-Induced Murine Colitis and T Helper 1 T-Cell Activation

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ABSTRACT

The proinflammatory cytokines interleukin (IL)-1β and IL-18 are supposed to play a crucial role in the pathogenesis of human inflammatory bowel disease. To exert biological activity, the precursors of both IL-1β and IL-18 need to be cleaved by the interleukin-1β-converting enzyme (ICE). IL-18 induces the synthesis of IFN-γ in T cells and NK cells. In the present study, we investigated the effect of the specific ICE inhibitor pralnacasan in dextran sulfate sodium-induced murine colitis. Colitis was induced in BALB/c mice by 3.5% dextran sulfate sodium dissolved in drinking water for 10 days. Pralnacasan was administered either intraperitoneally or orally every day. To assess in vivo efficacy, a clinical disease activity score was evaluated daily. Colon length, expression of IL-18 in colonic tissue, expression of interferon-γ (IFN-γ) in paraaortal lymphocytes, and systemic production of IFN-γ in splenocytes were analyzed post mortem. Intraperitoneally administered pralnacasan significantly reduced the clinical score compared with the dextran sulfate sodium control group from day 6 to day 10. Oral administration of pralnacasan also significantly reduced the clinical score at days 8 and 9. Administration of pralnacasan i.p. reduced the expression of intracolonic IL-18 significantly. Furthermore, pralnacasan reduced the number of IFN-γ-positive lymphocytes in paraaortal lymph nodes. IFN-γ synthesis in stimulated splenocytes was significantly suppressed in all pralnacasan-treated groups. No side effects of pralnacasan were observed. In conclusion, pralnacasan is effective in the prevention of dextran sulfate sodium-induced colitis. This effect is probably mediated by suppression of the proinflammatory cytokines IL-18, IL-1β, and IFN-γ.

Cytokines orchestrate the pathogenesis of inflammatory bowel disease. Successful treatment of patients with steroid-refractory Crohn’s disease (CD) with anti-TNF-α antibody (van Dullemen et al., 1998; Rutgeerts et al., 1999; Sandborn and Hanauer, 1999) illustrates the potency in treating CD based on the specific blockade of this proinflammatory cytokine (for review, see Eigler et al., 1997). The specific blockade of type IV phosphodiesterase with mesopram decreases the kine (for review, see Eigler et al., 1997). The specific blockade and Hanauer, 1999) illustrates the potency in treating CD (van Dullemen et al., 1998; Rutgeerts et al., 1999; Sandborn and Hanauer, 1999). Thus, in the treatment of human inflammatory bowel disease (IBD) further strategies to antagonize proinflammatory cytokines are warranted.

IL-1β is produced by activated monocytes and macrophages. It stimulates B and T lymphocytes as well as bone marrow cells, induces the acute phase reaction, and acts as an endogenous pyrogen (Dinarello, 1996). Mononuclear cells isolated from colon specimens from patients with ulcerative colitis or CD produce more IL-1β compared with cells from...
normal mucosa, providing circumstantial evidence for an active role of IL-1β in human IBD (Mahida et al., 1989).

IL-18 is structurally related to IL-1β; both are all-β-sheet protein precursors. In contrast with IL-1β, IL-18 mRNA and precursor protein are constitutively expressed in human peripheral blood mononuclear cells as well as in murine splenocytes and keratinocytes (Stoll et al., 1997). The production of biologically active IL-18 is induced by different stimuli such as bacterial lipopolysaccharides or cytokines such as TNF-α or IL-1β. IL-18 induces the production of IFN-γ in T cells and NK cells (Dinarello, 1999). It acts synergistically with IL-12, which up-regulates IL-18 receptors in target cells. In BALB/c mice, induction of the expression of IFN-γ by IL-18 leads to a shift to a Th1 response of the immune system (Szabo et al., 1997). This shift is thought to be relevant in the pathogenesis of human CD and might also contribute to murine colitis induced by dextran sulfate sodium as an enhancing factor. Both IL1-β and IL-18 are produced as biologically inactive precursor proteins that require cleavage by the interleukin-1β converting enzyme (ICE), which is a member of the family of aspartate-specific cysteine proteases (caspases). ICE is produced as a 45-kDa proenzyme that lacks proteolytic activity and is present in mononuclear cells (Ayala et al., 1994). Upon cell stimulation, proenzymes aggregate and subsequently undergo autocatalytic activation that presumably leads to a tetramer consisting of two 10-kDa and two 20-kDa polypeptide fragments of ICE with the active site Cys-285 localized near to the C terminus of the 20-kDa peptide chain (Wilson et al., 1994; Gu et al., 1995; Schonbeck et al., 1997). Active ICE prevails in the plasma membrane where it cleaves pro-IL-1β and pro-IL-18. It is complexed with other proteins that are involved in the secretion of the activated cytokines (Singer et al., 1995).

Several classes of ICE inhibitors exist, which can be divided into irreversible and reversible inactivators of this protease (Livingstone et al., 1997). Irreversible inactivators react with the active site sulfhydryl group in the manner of a nucleophile substitution and therefore must provide leaving groups in their chemical structure. Such inhibitors belong chemically to the halomethylketones, diazomethylketones, or acyloxymethylketones. Reversible inactivators of ICE are phenylalkylketones and peptide aldehydes, which have a high affinity for binding to the enzyme and thus are also highly effective protease inhibitors (Thornberry et al., 1994; Livingstone et al., 1997).

Dextran sulfate sodium-induced colitis is characterized histopathologically by mucosal infiltration of inflammatory cells, focal crypt damage, epithelial injury, and ulceration (Cooper et al., 1993; Dieleman et al., 1997; Okayasu et al., 2002). The pathological mechanism of dextran sulfate sodium-induced colitis includes both toxic effects on the epitheliun and production of proinflammatory cytokines by macrophages that are activated after phagocytosis of dextran sulfate sodium. The purpose of this study was to investigate the disease-modifying activity of the ICE inhibitor pralnacasan (previously known as HMR 3480/VX-740) in a murine model of dextran sulfate sodium-induced colitis (Siegmund and Zeitz, 2003). Pralnacasan is an ethyl-hemiacetal orally bioavailable prodrug. Once absorbed, it is rapidly hydrolyzed to the active but reversible aldehyde protease inhibitor (M. D. Mullican and D. J. Lauffer, manuscript submitted for publication). The active compound AcYVAD-CHO was structure based designed using crystallographic structural data to model the interaction between ICE and the acetylated tetrapeptide ICE inhibitor AcYVAD-CHO (Siegmund and Zeitz, 2003).

### Materials and Methods

**Mice.** Female, 8-week-old BALB/c mice (Harlan Winkelmann, Borehen, Germany) weighing 20 to 22 g were housed in temperature-controlled rooms with a 12-h light/dark cycle. Mice were fed standard mice chow pellets, had access to bottled tap water ad libitum, and were acclimatized to the environmental conditions 10 days before they were studied in the experiment. Mice were killed by cervical dislocation under isoflurane anesthesia (Forene; Abbott GmbH, Wiesbaden, Germany). All experiments were approved by the regional animal study committee and are in agreement with the guidelines for the proper use of animals in biomedical research. Both animal handling and clinical and histological scoring of colitis were performed in a blinded experimental design.

**Reagents.** Brefeldin A, phosphol 12-myristate 13-acetate (PMA), and ionomycin were purchased from Sigma Chemie (Munich, Germany). RPMI 1640 medium was from Biochrom KG (Berlin, Germany) and fetal calf serum (FCS) was from Invitrogen (Karlsruhe, Germany). For i.p. use, pralnacasan was dissolved in 25% Cremophor EL solution and filtered through syringe filters (0.2 μm) purchased from Gelman Sciences (Ann Arbor, MI). For p.o. use, pralnacasan was homogenously suspended in 0.5% hydroxyethylcellulose solution (HEC, lot S29068015; HEC, Schuchard, Hohenbrunn, Germany).

**Induction of Colitis and Treatment.** Mice were fed 3.5% dextran sulfate sodium (lot 6457 C, molecular mass 30–40 kDa; ICN, Eschwege, Germany) dissolved in sterile, distilled water ad libitum throughout the experiment (days 1–10) as described previously (Hartmann et al., 2000; Siegmund et al., 2001c; Okayasu et al., 2002). Pralnacasan was administered twice daily intraperitoneally at a dose of 50 mg/kg body weight as well as orally at a dose of 50 or 12.5 mg/kg body weight, respectively. Placebo-treated animals received 0.5% HEC solution orally twice daily. Control mice were fed with tap water and received twice daily pralnacasan (50 mg/kg body weight) either intraperitoneally or orally. The dosage volume was 200 μl for both intraperitoneal and oral administration. For the experimental protocol, also see Table 1.

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**Table 1**

Experimental protocol to investigate the efficacy of pralnacasan in DSS-induced colitis

<table>
<thead>
<tr>
<th>Group</th>
<th>Solvent</th>
<th>Number</th>
<th>Administration Route</th>
<th>Pralnacasan (mg/kg)</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>DSS + HEC</td>
<td>0.5% HEC</td>
<td>8</td>
<td>p.o.</td>
<td>0</td>
<td>1/8</td>
</tr>
<tr>
<td>DSS + pralnacasan</td>
<td>25% cremophor EL</td>
<td>8</td>
<td>i.p.</td>
<td>50 b.i.d.</td>
<td>0/8</td>
</tr>
<tr>
<td>DSS + pralnacasan</td>
<td>0.5% HEC</td>
<td>8</td>
<td>p.o.</td>
<td>12.5 b.i.d.</td>
<td>3/8</td>
</tr>
<tr>
<td>DSS + pralnacasan</td>
<td>0.5% HEC</td>
<td>8</td>
<td>p.o.</td>
<td>50 b.i.d.</td>
<td>0/8</td>
</tr>
<tr>
<td>Control + pralnacasan</td>
<td>25% cremophor EL</td>
<td>2</td>
<td>i.p.</td>
<td>50 b.i.d.</td>
<td>0/2</td>
</tr>
<tr>
<td>Control + pralnacasan</td>
<td>0.5% HEC</td>
<td>2</td>
<td></td>
<td>50 b.i.d.</td>
<td>0/2</td>
</tr>
</tbody>
</table>
Clinical Score. Body weight, occult blood, or the presence of gross blood per rectum, and stool consistency were determined daily as described previously (Dieleman et al., 1997; Hartmann et al., 2000; Siegmund et al., 1999). Two investigators blinded to the treatment groups assessed the clinical score: weight loss <1% was counted as 0 points, weight loss of 1 to <5% as 1 point, 5 to <10% as 2 points, 10 to 20% as 3 points, and more than 20% as 4 points. For stool consistency, 0 points were given for well formed pellets; 2 points for pasty and semiformal stools, which did not stick to the anus, and 4 points for liquid stools that did stick to the anus. Bleeding was scored 0 points for no blood in hemoccult; 2 points for positive hemoccult; and 4 points for gross bleeding. The average of these three scores (body weight, stool consistency, and rectal bleeding) gave an overall clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis).

Colon Length and Histological Score. Post mortem, the entire colon was removed from the cecum to the anus and the colon length was measured as an indirect marker of inflammation. Rings of the transverse part of the colon were fixed in 10% formalin and embedded in paraffin for blinded histological analysis. Sections were stained with hematoxylin/eosin. Histological scoring was performed in a blinded way by a pathologist as described previously (Hartmann et al., 2000; Siegmund et al., 2001c). For infiltration of inflammatory cells, rare inflammatory cells in the lamina propria were counted as 0, increased numbers of inflammatory cells in the lamina propria as 1, confluent of inflammatory cells extending into the submucosa as 2, and transmural extension of the infiltrate as 3. For tissue damage, no mucosal damage was counted as 0, discrete lymphoepithelial lesions as 1, surface mucosal erosion as 2, and extensive mucosal damage and extension through deeper structures of the bowel wall as 3. The two subscores (cell infiltration and tissue damage) were added and the combined histological score ranged from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).

All animals that were alive at the end of the treatment interval were included in the evaluation scores. Mice that died during the treatment period represent a population of maximally ill mice, and these animals were assigned a clinical score of 4 (maximally ill). However, these animals were excluded from the post mortem and ex vivo parameters.

Synthesis of Intracolonic IL-18. To study colon homogenate at the end of the experimental course, colons were removed and strips of the colon (about 0.7 cm) were mechanically crushed, vigorously vortexed for 1 min in 200 μl of tissue protein extraction reagent (Pierce Chemical, Rockford, IL), and shocked in liquid nitrogen. The homogenate was centrifuged at 10,000 g for 15 min. The amount of total extracted protein was determined by Bradford analysis using Bio-Rad protein assay (Bio-Rad, Munich, Germany) as dye reagent. The amount of IL-18 in the colon homogenate was quantified by ELISA (BD Pharmingen, San Diego, CA) according to the manufacturer's instructions.

Lymphocyte Cell Culture. At day 11, paraaortal lymph nodes were removed aseptically, crushed mechanically, and cell suspensions were prepared as described previously (Coligan et al., 1992). Cells were washed and resuspended in RPMI 1640 medium containing 10% FCS, PMA (10 ng/ml), ionomycin (500 ng/ml), and brefeldin A (1 μg/ml) and cultured at a concentration of 2.5 × 10^6/ml in 48-well plates at 37°C in a humidified atmosphere with 5% CO₂. After 5 h, cells were harvested to perform FACs analysis.

Spleen Cell Culture. At day 11, spleens were removed aseptically, weighed, and cell suspensions were prepared according to the standard procedures (Coligan et al., 1992): Spleens were pressed through a 100-μm nylon cell strainer (Falcon, Cowley, UK), washed, and erythrocyte lystate solution (lot L241; Ortho Clinical Diagnostics GmbH, Neckargemünd, Germany) was added. Cells were incubated for 12 min, washed twice in RPMI 1640 medium, resuspended in medium containing 10% FCS, and cultured at 2.5 × 10^6/ml in 48-well plates. Cultures were incubated for 20 h in the presence of PMA (10 ng/ml) plus ionomycin (500 ng/ml) at 37°C in a humidified atmosphere with 5% CO₂. The incubation period was terminated by freezing the plates at −70°C. To obtain combined lysate plus supernatant, the samples were exposed to three freeze-thaw cycles before determining intracellular cytokine concentration. IFN-γ was quantified by ELISA (Endogen, Woburn, MA).

Flow Cytometry. Flow cytometry followed routine procedures using 2 × 10⁶ lymphocytes per sample. For the determination of CD69 expression, cells were labeled with a fluorescein isothiocyanate-labeled antibody (lot M044628; BD Biosciences, Heidelberg, Germany). Furthermore, lymphocytes were exposed to fixation medium and permeabilization medium (An der Grub, Kaumberg, Austria) and were intracellularly stained with a phycoerythrin-labeled anti-mouse IFN-γ antibody (lot M051507; BD Biosciences). Samples were analyzed by a FACSCalibur (BD Biosciences).

Statistical Analysis. Data are expressed as means ± S.E.M. Statistical significance of differences between treatment and control groups was determined by factorial analysis of variance. Differences were considered statistically significant for p < 0.05. Statistical analyses were performed using StatView 4.51 software (Abacus Concepts, Calabasas, CA).

Results

Effect of Pralnacasan Treatment on Clinical Scores in Dextran Sulfate Sodium Colitis. Pralnacasan was administered i.p. or p.o. twice daily at a dose of 50 mg/kg to dextran sulfate sodium and control groups from day 1 to 10 (n = 8 animals/group). An additional dextran sulfate sodium group was treated p.o. with 12.5 mg/kg pralnacasan twice daily (n = 8). Placebo-treated dextran sulfate sodium-exposed animals received 0.5% HEC solution twice daily from day 1 to 10 (n = 8). Mice fed dextran sulfate sodium developed colitis expressed by a clinical score of >0.5 beginning on day 4 (Fig. 1) and differed significantly from the control group from day 6 to day 10. Neither of the two nondextran sulfate sodium control groups showed signs of colitis. Intrapерitoneal administration of 50 mg/kg b.i.d. pralnacasan significantly reduced the clinical score in dextran sulfate sodium-exposed groups compared with placebo-treated mice from day 6 (score 0.0 ± 0.0 versus 1.6 ± 0.3; p < 0.001) to day 10 (score 1.2 ± 0.3 versus 2.6 ± 0.5; p = 0.038). Oral administration of 50 mg/kg b.i.d. pralnacasan also reduced the extent of colitis. The difference of the clinical score of this dextran sulfate sodium-exposed group compared with the placebo-treated dextran sulfate sodium-exposed group was statistically significant on day 8 (score 1.3 ± 0.2 versus 2.4 ± 0.4; p = 0.034) and day 9 (score 1.5 ± 0.2 versus 2.7 ± 0.5; p = 0.046) but missed significance on day 10. There was no relevant difference between the clinical score of the dextran sulfate sodium-exposed group that was treated with 12.5 mg/kg b.i.d. pralnacasan p.o. and the dextran sulfate sodium-exposed group that received placebo. In the group treated with 12.5 mg/kg b.i.d. pralnacasan p.o., three mice died during the experiment (one mouse at day 8 without signs of colitis, albeit a substantial weight loss of 35% and two mice at day 10 with marked colitis). In the placebo-treated group, one mouse died from colitis at day 9.

Effect of Pralnacasan Treatment on Colon Length. Dextran sulfate sodium treatment led to a reduction of colon length in exposed mice. As shown in Fig. 2 pralnacasan partially reversed dextran sulfate sodium-induced colon shortening. Mean colon length in all pralnacasan-treated dextran sulfate sodium-exposed groups was significantly longer than in the placebo group (12.6 ± 0.7 cm in 50 mg/kg
b.i.d. i.p.-treated mice, \( p = 0.003; \) 11.1 ± 0.6 cm in 12.5 mg/kg b.i.d. p.o.-treated mice, \( p = 0.047; \) 11.0 ± 0.5 cm in 50 mg/kg b.i.d. p.o.-treated mice, \( p = 0.022 \) versus 9.1 ± 0.5 cm in placebo-treated mice).

**Histological Score.** Histological analysis of rings of the transverse part of the colon was performed by a pathologist blinded to the protocol. All dextran sulfate sodium-exposed groups showed histological signs of inflammation (Fig. 3), whereas the colons of the control groups revealed no signs of inflammation (0.0 ± 0). The group that was intraperitoneally treated with 50 mg/kg b.i.d. pralnacasan showed a trend (\( p = 0.204 \)) to an improved histological score (2.5 ± 0.7, compared with the other dextran sulfate sodium-exposed groups (3.8 ± 0.6 in the placebo group, 3.8 ± 0.8 in the 12.5 mg/kg b.i.d. p.o. group and 3.6 in the 50 mg/kg b.i.d. p.o. group).

**Synthesis of Intracolonic IL-18.** IL-18 was quantified in the colonic homogenate by ELISA. As shown in Fig. 4, non-dextran sulfate sodium-exposed groups showed the lowest level of colonic IL-18 concentration (152 ± 40 pg/mg protein, \( n = 4 \) in the pralnacasan-treated control group), dextran sulfate sodium treatment led to a markedly increased content of IL-18 (317 ± 76 pg/mg protein, \( n = 5 \) animals in the HEC-treated group). As shown in Fig. 4, administration of 50 mg/kg b.i.d. pralnacasan i.p. reduced intracolonic IL-18 content significantly (\( p = 0.016 \)) compared with the placebo-treated group (113 ± 22 pg/mg protein, \( n = 8 \), in the i.p. pralnacasan-treated dextran sulfate sodium-receiving group).

**Production of IFN-γ by Lymphocytes of Paraaortal Lymph Nodes.** We also evaluated the production of the proinflammatory Th1 cytokine IFN-γ by lymphocytes harvested from paraaortal lymphnodes, which are the draining lymphatic tissue of the colon. Exposure to dextran sulfate sodium led to an increase of the number of IFN-γ-producing cells in the draining lymph nodes of the colon (Fig. 5). In the placebo-treated group 84 ± 2% of the lymphocytes isolated from paraaortal lymph nodes were IFN-γ positive (\( n = 4 \)) compared with 72 ± 1% (\( n = 4 \)) in the control group, which was not exposed to dextran sulfate sodium. Prevention of this increase by treatment with pralnacasan reflects the results obtained by the clinical score. Treatment with 12.5 mg/kg b.i.d. pralnacasan showed no reduction in the number of IFN-γ positive lymphocytes (82 ± 2%, \( n = 4 \)), whereas oral treatment with 50 mg/kg b.i.d. pralnacasan decreased this...
number to 79 ± 4% (n = 4). Intraperitoneal treatment with 50 mg/kg b.i.d. pralnacasan reduced IFN-γ-positive lymphocytes statistically markedly to 75 ± 1% (n = 3).

**In Vitro Synthesis of IFN-γ by Splenocytes.** To evaluate the systemic effect of pralnacasan on the suppression of IFN-γ synthesis, splenocytes harvested from mice at the end of the experimental course were stimulated with PMA (10 ng/ml) and ionomycin (1 µg/ml). After a 20-h incubation period, cells were processed as described under Materials and Methods and IFN-γ was quantified by ELISA.

Pralnacasan treatment suppressed IFN-γ synthesis by splenocytes in all treated groups (Fig. 6). Only the placebo-treated group showed a substantially increased production of IFN-γ after stimulation with PMA and ionomycin (5721 ± 1506 pg/ml, n = 7). The difference in IFN-γ production between the placebo-treated group and all pralnacasan-treated groups was statistically significant (12.5 mg/kg b.i.d. pralnacasan p.o. 689 ± 84 pg/ml, n = 5, p = 0.006; 50 mg/kg b.i.d. pralnacasan p.o. 1172 ± 110 pg/ml, n = 8, p = 0.007; and 50 mg/kg b.i.d. pralnacasan i.p. 778 ± 175 pg/ml, n = 8, p = 0.004).

**Discussion**

The present study is the first to demonstrate the efficacy of an ICE inhibitor in a colitis model. Mice were exposed to 3.5% dextran sulfate sodium in the drinking water and developed colitis that was quantified by a clinical score. Post mortem colon length was quantified, and histological analysis of strips of the colon was performed by a pathologist. The con-
tent of intracolonic cytokines was measured by ELISA. Furthermore, the synthesis of IFN-γ was quantified in paraaortal lymph nodes and in cultured splenocytes stimulated with PMA. All of the mentioned study endpoints were evaluated regarding the therapeutic efficacy of pralnacasan. Especially, the clinical score and the cytokine measurement revealed that dextran sulfate sodium-exposed animals derived a marked benefit from treatment with the ICE inhibitor pralnacasan.

Treatment with pralnacasan dose dependently improved the clinical signs of the colitis. Oral application of 50 mg/kg b.i.d. pralnacasan led to a clear benefit evidenced by a reduction of clinical signs of colitis, whereas 12.5 mg/kg b.i.d. pralnacasan had no effect on this parameter. The substance was even more effective when administered intraperitoneally. For the latter case, the difference to the placebo group was significant from day 6 to the end of the experimental course. Previous experiments on dextran sulfate sodium-induced colitis (data not shown) have demonstrated that there is no influence of cremophor EL administered intraperitoneally, neither on the clinical course of dextran sulfate sodium-induced colitis nor on the expression of cytokines. In these experiments, mice that received dextran sulfate sodium and NaCl i.p. showed the same clinical score and equivalent levels of proinflammatory cytokines as mice that received dextran sulfate sodium and cremophor EL i.p. Therefore, p.o. administration of HEC, the solvent of pralnacasan for p.o. use, seemed more reasonable as a placebo for the experiment investigating the therapeutic efficacy of pralnacasan. We decided to use the orally administered HEC as placebo control, because the mode of oral administration of a drug bears the risk of injuring the animal with consequent upper gastrointestinal bleeding, resulting in a positive hemoccult test.

Reduction of colon length is a well established post-mortem...
surrogate parameter that correlates with the extent of the colitis (Okayasu et al., 2002; Hartmann et al., 2000; Siegmund et al., 2001c). Placebo-treated dextran sulfate sodium-exposed mice showed a marked reduction of colon length compared with nondextran sulfate sodium-exposed mice. Pralnacasan partially prevented the shortening of the colon even at the oral dose of 12.5 mg/kg, which did not reduce the clinical score. Again, the intraperitoneal route of application was superior to oral administration of the same amount. The benefit of 50 mg/kg b.i.d. orally administered pralnacasan on this endpoint compared with placebo or the 12.5 mg/kg b.i.d. dose surely is blunted, because in the placebo group one mouse and in the 12.5 mg/kg b.i.d. pralnacasan group three mice died during the experiment and were not evaluated concerning this parameter.

The Th1-mediated immune response seems to play a key role in inflammatory bowel disease. Active IL-18 is highly effective in inducing the expression of the Th1 cytokine IFN-γ (Dinarello, 1999). Thus, high levels of IL-18 in the dextran sulfate sodium-receiving placebo group indicate that the activation of the Th1 pathway might contribute to the pathogenesis of dextran sulfate sodium-induced murine colitis. ICE is necessary for the activation of IL-18. Cleavage of pro-IL-18 is necessary for the release of mature IL-18 from the intracellular compartment by ICE, which is an intracellular enzyme (Dinarello, 2000). In fact, we found significantly reduced levels of IL-18 in colon homogenates of mice treated with the ICE inhibitor pralnacasan. The finding that IL-18 levels were not reduced in the p.o.-treated mice is consistent with our clinical observation that i.p. administration of pralnacasan is more effective than p.o. treatment. Pralnacasan not only reduces the cleavage of interleukin-18 but also of interleukin-1β. A disturbed balance between IL-1β and its physiological inhibitor IL-1 receptor antagonist (IL-1ra) seems to represent an important pathogenic factor of inflammatory diseases such as rheumatoid arthritis (Horai et al., 2000). Therefore, inhibition of ICE by pralnacasan might exert a dual antiinflammatory effect, reducing the proinflammatory action of IL-18 and IL-1β.

Inhibition of the cleavage of pro-IL-18 should result in a suppression of IFN-γ expression. In the present study, we investigated the production of IFN-γ in lymphocytes harvested from paraaortal lymph nodes that are the draining lymph nodes of the colon. We showed that significantly more lymphocytes were IFN-γ positive in dextran sulfate sodium-exposed mice treated with placebo than in control animals receiving no dextran sulfate sodium. Furthermore, there was a dose-dependent reduction of IFN-γ-positive lymphocytes in pralnacasan-treated mice that reflected the clinical score. Moreover, we provide evidence for a systemic anti-inflammatory effect of pralnacasan. Splenocytes harvested from mice that were not treated with the ICE inhibitor produced high amounts of IFN-γ upon stimulation. This ex vivo synthesis was suppressed in all pralnacasan-treated groups.

Our experimental findings are consistent with the findings of Siegmund et al. (2001b) who could demonstrate a reduced susceptibility of ICE KO mice to dextran sulfate sodium-induced colitis. Flow cytometric analysis of isolated mesenteric lymph node cells showed reduced cell activation in ICE KO mice. We could previously show that anti-IL-18 treatment resulted in a dose-dependent reduction of the severity of dextran sulfate sodium-induced colitis (Siegmund et al., 2001a). In the colon tissue homogenates, IFN-γ concentrations were lower in the anti-IL-18-treated dextran sulfate sodium-fed mice compared with untreated dextran sulfate sodium-fed mice. Consistently, we found elevated levels of intracolonic IL-18 after dextran sulfate sodium administration. Recently, ten Hove et al. (2001) demonstrated the amelioration of trinitrobenzene sulfonic acid-induced colitis by blockade of endogenous IL-18 by a recombinant human IL-18-binding protein. In the experiments reported by her group, reduced levels of TNF-α, IL-1β, and IL-6 were measured in colon homogenates of IL-18-binding protein-treated animals (Ten Hove et al., 2001).

Our results are also concordant with findings of Hans et al. (2000) who demonstrated a deterioration of dextran sulfate sodium-induced colitis by treatment of mice with murine IL-12 during induction of colitis. This effect was mediated by an increased production of IFN-γ and was completely antagonized by simultaneous application of anti-IFN-γ antibodies. Yet, anti-IL-12 antibodies only had weak ameliorating effects on the course of normal dextran sulfate sodium-induced colitis without additional application of IL-12 (Hans et al., 2000). Together, the results of these studies demonstrate that production of IL-18 seems to be one of the key mechanisms in dextran sulfate sodium-induced colitis. This cytokine induces the production of IFN-γ which may be enhanced by additional application of IL-12. Finally, this pathway results in a Th-1-mediated immune response (Siegmund, 2002). Recent data (data not shown) indicate that pralnacasan is able to suppress the up-regulation of proinflammatory cytokines on the mRNA and on the protein level. Investigations on the effect of pralnacasan in animal models of inflammatory bowel disease other than the dextran sulfate sodium model are necessary (Siegmund, 2002). IL-10-deficient mice and transfer models of IBD might be a tool to further elucidate the pathophysiological role of ICE inhibition by pralnacasan.

We conclude that pralnacasan significantly attenuates dextran sulfate sodium-induced murine colitis. The key pathway of this treatment may be the inhibition of IL-18 activation, which prevents the induction of IFN-γ. This inhibits the Th-1-mediated immune response. A clinical pilot study to determine the efficacy of pralnacasan in patients with Crohn’s disease is warranted.

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References


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