Specific Type IV Phosphodiesterase Inhibitor Rolipram Mitigates Experimental Colitis in Mice

GUNTHE HARTMANN, CHRISTOPH BIDLINGMAIER, BRITTA SIEGMUND, STEFAN ALBIRICH, JOHANNES SCHULZE, KATHARINA TSCHOEP, ANDREAS EIGLER, HANS ANTON LEHR, and STEFAN ENDRES

Division of Clinical Pharmacology, Medizinische Klinik, Klinikum Innenstadt, University of Munich, Germany

Accepted for publication September 13, 1999 This paper is available online at http://www.jpet.org

ABSTRACT

The specific type IV phosphodiesterase inhibitor rolipram is a potent suppressor of tumor necrosis factor-α (TNF) synthesis. We examined the efficacy of rolipram for the prevention and treatment of experimental colitis. To induce colitis, BALB/c mice received 5% dextran sulfate sodium in their drinking water continuously for up to 11 days. Colitis was quantified by a clinical activity score assessing weight loss, stool consistency, and rectal bleeding (range from 0 to 4); by colon length; and by detecting TNF concentration in colonic tissue by enzyme-linked immunosorbent assay. In a first protocol, rolipram (10 mg/kg b.wt./day i.p.) was started on the same day as dextran sulfate sodium exposure was discontinued on day 7 and rolipram was administered from day 8 through day 15. These three series of experiments on a total of 153 mice documented the efficacy of rolipram in both the prevention and treatment of experimental colitis.

In several diseases, the proinflammatory cytokine tumor necrosis factor-α (TNF) forms a necessary element in the chain of pathophysiologic events leading to inflammation. Successful treatment with anti-TNF-antibodies in patients with Crohn’s disease (van Dullemen et al., 1995; Stack et al., 1997; Targan et al., 1997; Present et al., 1999), with rheumatoid arthritis (Elliott et al., 1994), and with Jarisch-Herxheimer reaction (Fekade et al., 1996) illustrate anti-inflammatory strategies based on the specific blockade of TNF (Eigler et al. 1997). Among the agents known to inhibit TNF production rather than block its function, attention has focused on cAMP-elevating phosphodiesterase (PDE) inhibitors. The predominant PDE isoenzyme family in monocytes, a main source of TNF production, is the PDEs of type IV.

Rolipram has initially been developed and studied in clinical trials as an antidepressant (Wachtel 1983). Recently, the potential therapeutic use of rolipram in TNF-dependent disease has been demonstrated in several animal models. Rolipram mitigated experimental autoimmune encephalomyelitis in rats (Sommer et al., 1995) and in nonhuman primates (Genain et al., 1995) and decreased clinical activity of experimental arthritis in rats (Nyman et al., 1997; Ross et al., 1997). In mice, rolipram decreased lipopolysaccharide-induced TNF plasma levels (Fischer et al., 1993) and protected from T-cell-mediated liver failure (Gantner et al., 1997).

In humans, the majority of inflammatory bowel disease occurs in two related, albeit clinically and histologically distinct disorders, ulcerative colitis and Crohn’s disease. Both diseases are characterized by chronically relapsing inflammation of the bowel of unknown cause. Crohn’s disease is characterized by a granulomatous, transmural inflammation of the bowel wall, predominantly in the distal ileum. In contrast, ulcerative colitis is defined by crypt abscesses and ulcerations limited to mucosa and submucosa, associated

ABBREVIATIONS: TNF, tumor necrosis factor-α; PDE, phosphodiesterase; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; IL, interleukin.
with a prominent inflammatory infiltrate. The mainstay of therapy for inflammatory bowel disease is aminosalicylates and topical and systemic glucocorticoids. Both provide therapeutic benefit and improve quality of life in many patients, but others suffer from recurrent disease despite systemic glucocorticoid therapy. More specific and more effective therapeutic agents with fewer side effects are needed for the permanent control of inflammatory bowel disease.

Several experimental models of inflammatory bowel disease have been described (Kim and Berstad, 1992; Dielemann et al., 1994; Elson et al., 1995). The dextran sulfate sodium (DSS) model of colitis has been recommended for preclinical testing of new pharmacologic compounds for therapy of chronic inflammatory bowel disease (Cooper et al., 1993; Elson et al., 1995). DSS-induced colitis has a number of advantages, including its simplicity, the ability to induce inflammatory lesions, and the reproducibility in respect to both time course and severity among individual mice of a given inbred strain. As in Crohn’s disease, macrophage activation and TNF production play a key role in DSS-induced colitis. Elevated levels of TNF have been found in the inflamed colons of DSS-treated mice (Dielemann et al., 1994).

Several studies pointed to a necessary mediator function of TNF, particularly in Crohn’s disease (Targan et al., 1997; van Deventer, 1997; Present et al., 1999). TNF elevation was more pronounced in Crohn’s disease than in ulcerative colitis both in plasma (Murch et al., 1991) and mucosal samples (Murch et al., 1993; Breese et al., 1994). In the present article, we extend our previous in vitro studies on TNF inhibition by rolipram (Semmler et al., 1993; Siegmund et al., 1997; Eigler et al., 1998) to demonstrate that rolipram attenuates the development of experimental colitis and improves recovery of animals with established colitis.

Materials and Methods

Animals and Induction of Colitis. Female BALB/c mice (~6 weeks of age, mean body weight 20 g) were purchased from Harlan Winkelmann GmbH (Borchen, Germany). Mice were kept under standard laboratory conditions at the animal facility at the Medizinische Klinik, Klinikum Innenstadt. Drinking water and food were provided ad libitum. Mice were sacrificed by cervical dislocation under isoflurane anesthesia (Forene; Abbott GmbH, Wiesbaden, Germany). All experiments were approved by the regional animal protection committee and are in agreement with the guidelines for the proper use of animals in biomedical research. Animal handling and scoring of colitis were performed in a consequently blinded experimental design.

DSS (molecular mass 40,000 Da) was obtained from ICN Biomedicals GmbH (Eschwege, Germany) and dissolved in distilled water. Colitis was induced by providing drinking water containing 5% DSS (w/v) for 7 to 11 days as indicated. Control mice received distilled water.

Clinical Activity Score. Colitis was quantified with a clinical score assessing weight loss, stool consistency, and bleeding (measured by guaiac reaction, hemocult) as described previously (Cooper et al., 1993). No weight loss 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 >20% as 4 points. For stool consistency, 0 points were given for well formed pellets, 2 points for pasty and semifomed stools that 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 hemocult, 2 points for positive hemocult, and 4 points for gross bleeding. These scores were added and divided by 3, forming a total clinical score that ranged from 0.0 (healthy) to 4.0 (maximal activity of colitis).

Colon Length, Histologic Scoring, and Mean Cross-Sectional Area. Postmortem, the entire colon was removed from the cecum to the anus and placed without tension on cellulose. Colon length was measured as an indirect marker of inflammation. Rings of the ascending, transverse, and descending part of the colon were fixed in 10% formalin and embedded in paraffin for histologic analysis. Sections were stained with hematoxylin/eosin. Histologic scoring was performed by a pathologist (0 to 3 points for infiltration of inflammatory cells plus 0 to 3 points for the degree of tissue damage). 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; confluence of inflammatory cells, extending into the submucosa as 2; and a score of 3 was given for transmural extension of the infiltrate. For tissue damage, no mucosal damage was counted as 0, discrete lymphoepithelial lesions were counted as 1, surface mucosal erosion was counted as 2, and a score of 3 was given for extensive mucosal damage and extension through deeper structures of the bowel wall. The combined histologic score ranged from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).

For the image analysis of cross-sectional areas, glass slides were imported into Photoshop (Adobe Systems Incorporated, San Jose, CA) on an Apple computer (G3; Apple Computer, Inc., Cupertino, CA) with a kodachrome slide scanner (Nikon LS 1000). Kodachrome frames were opened on one side to allow introduction of the glass slide. Three entire cross sections of each colon part were selected with the “magic wand tool” in the Photoshop toolbox (Lehr et al., 1997). The cross-sectional area of the three bowel sections was quantified with the “histogram tool” (in number of pixels) and was transformed into square millimeters with comparative measurements of a micrometer slide.

Treatment with Rolipram. Rolipram (0.5 mg), kindly supplied by Schering AG (Berlin, Germany), was diluted in 1 ml of distilled water by heating the solution for 30 s at 60°C and then cooling it at room temperature for 2 min. This was repeated until rolipram was totally dissolved. The solution was then frozen into aliquots of 2 ml at ~80°C. This proved to be the most reliable protocol to dissolve a relatively high concentration of rolipram for a low total injection volume of 200 μl. Rolipram (5 mg/kg b.wt. b.i.d.) was injected twice per day i.p. with a total injection volume of 200 μl each. Control mice were injected with 200 μl of 0.9% NaCl. To test the therapeutic efficacy of rolipram, two protocols were used: 1) in the concurrent treatment protocol (prevention of colitis), DSS was administered for up to 11 days with rolipram therapy starting the same day as DSS; and 2) in the delayed treatment protocol (treatment of established colitis), colitis was first induced by DSS administration from day 1 to day 7, rolipram therapy was then started at day 8, and was continued up to day 15.

Colon Cytokine Extraction. Strips (~4 cm) of colon from DSS-exposed mice with or without rolipram treatment were weighed, vigorously vortexed for 1 min in 100 μl of 0.01 M PBS (Boehringer Mannheim, Ingelheim, Germany), and centrifuged at 10,000g at 4°C for 15 min. TNF was quantified in the eluate with a commercial enzyme-linked immunosorbent assay (ELISA) kit (Endogen, Woburn, MA) according to the manufacturer’s instructions. The lower limit of detection of the assay is 50 pg/ml.

Statistical Analysis. All data are expressed as means ± S.E. Statistical significance of differences between treatment and control groups was determined by the unpaired two-tailed Student’s t test. Where applicable, P values were corrected by the Bonferroni method for three independent comparisons (clinical score, colon length, and histologic degree of inflammation). Differences were considered statistically significant for P < .050. Statistical analyses were performed with StatView 512 software (Abacus Concepts, Berkeley, CA).
Results

Characteristics of Colitis. Treatment of BALB/c mice with 5% DSS in drinking water for 7 or 11 days resulted in clinical, gross, and histologic signs of colitis that resolved gradually when DSS administration was discontinued (Figs. 1–5). Mice produced loose stool or diarrhea, occult or gross rectal bleeding, and lost weight. After 11 days, the colon length of DSS-treated mice was 12.4 ± 0.3 cm (n = 5) compared with 17.4 ± 0.3 cm (n = 4; P < .001) in healthy controls. This has been described as a morphologic parameter of colon inflammation (Okayasu et al., 1990).

Histologic examination of colon sections from control mice showed no signs of inflammation (Figs. 1A and 2A). Histology of colon sections of DSS-treated mice revealed multiple erosive lesions and inflammatory cell infiltration composed mainly of macrophages with fewer lymphocytes and occasional eosinophils and neutrophils (Fig. 1, B and C). In some colon sections, particularly of advanced lesions, regeneration of epithelium were noted in the mucosa. The histologic score (assessing the extent of infiltration of inflammatory cells and the degree of tissue damage, range from 0 to 6) was determined as the mean score of sections of the ascending colon, transverse colon, and descending colon, each section evaluated separately. The most severe lesions were observed in the transverse and the descending colon. After 11 days of continuous DSS administration, a histologic severity score of 4.6 ± 0.5 (n = 14) was reached (Fig. 6).

Mice studied at different time points during DSS treat-

Fig. 1. Histologic characteristics of DSS-induced colitis. A, normal colonic mucosa with regularly formed colonic folds covered by intact mucosa. Only occasional leukocytes are present in the lamina propria of the mucosa. B, a focal accumulation of lymphocytes, histiocytes, and fewer neutrophils compared with (A). Although the surface layer of the mucosa is still intact, there is marked crypt destruction by the inflammatory infiltrate. The inflammatory process is limited to the mucosa and submucosa. C, severe colonic inflammation, characterized by a dense inflammatory cell infiltrate in all colonic wall structures and widespread mucosal sloughing. Note the circumscription of the destructive process, immediately adjacent to normal appearing mucosa. Magnification of the images is 130-fold for (A) and (C), and 325-fold for (B).
ment (days 0 to 7) and after DSS discontinuation (days 8 to 15) developed increasing histologic signs of colitis over time (Fig. 3). Although mice improved clinically after discontinuation of DSS administration at day 7 (clinical score at day 8 was 2.1 (n = 2) compared with 0.8 at day 15 (n = 7; P = .008; data not shown), on the histologic level no decrease of cellular infiltration and tissue damage could be found until day 15 (Fig. 3). The apparent lack of improvement on the histologic level may be due to cellular infiltration involved in tissue regeneration during the first days of recovery.

Prevention of Colitis with Rolipram. In the concurrent treatment protocol, we tested the effect of rolipram on the prevention of DSS-induced colitis. Mice were administered 5% DSS in their drinking water and were injected i.p. with rolipram or with 0.9% NaCl for a total of 11 days. This protocol was studied in two independent experimental series. The first series comprised 14 mice. Mice fed with DSS developed clinical signs of colitis expressed by an activity score >0.5 starting from day 4 (Fig. 7). Intraperitoneal injection of rolipram in a dose of 10 mg/kg b.wt. daily did not retard onset of colitis during the first 6 days of DSS administration. After that, it significantly reduced the progression of colitis as expressed by a lower clinical activity score (1.1 ± 0.3; n = 5 in rolipram-treated mice compared with 2.4 ± 0.4; n = 5 in NaCl controls; P = .041; day 11) (Fig. 7). Each of the three clinical parameters assessed in the clinical score was beneficially influenced by rolipram (body weight, 19.5 g ± 1.5 g in rolipram-treated mice versus 18.0 ± 0.9 g in NaCl-treated controls; stool consistency score, 0.0 ± 0.0 versus 1.2 ± 0.5; rectal bleeding score, 0.8 ± 0.8 versus 2.4 ± 1.0). Control

**Fig. 2.** Influence of rolipram on the histologic manifestation of DSS-induced colitis. Shown are representative cross sections of the transversal colon in control mice without DSS (A) and in DSS-treated mice treated with i.p. injections of 0.9% NaCl (B) or rolipram [10 mg/kg b.wt., (C)] at day 11. Note the regional mucosal destruction in DSS-treated mice (B), which is effectively reversed by rolipram (C). In rolipram-treated mice, there is still some degree of edema, but no cross-mucosal damage. Also note the difference in colon cross-sectional area, which is partially reversed by rolipram. Magnification of the images is 52-fold.
mice (without DSS) treated with 0.9% NaCl or rolipram i.p. developed no signs of colitis (Fig. 7). At day 11, all mice were sacrificed. Of the DSS-treated mice, those with rolipram therapy had a longer colon (15.4 ± 0.7 cm) than NaCl controls (12.4 ± 0.3 cm; P = .004). Thus, rolipram had partially reversed the colon shortening induced by DSS compared with mice with normal drinking water (17.4 ± 0.3 cm). This argued for a lower extent of inflammation, which was confirmed by histologic examination in the rolipram group (Fig. 2C) compared with the NaCl group (Fig. 2B). In DSS-treated mice, rolipram decreased the histologic score compared with NaCl-treated control mice (1.5 ± 0.6 versus 4.6 ± 0.5; P = .020; Fig. 6).

The second experimental series studied with concurrent rolipram treatment comprised 36 animals. The beneficial effect of rolipram was confirmed by clinical (1.8 ± 0.6 in rolipram-treated mice (n = 14) versus 3.7 ± 0.3 in control mice (n = 14; P = .021; day 11; data not shown) and histologic scoring (1.9 ± 0.6 versus 4.3 ± 0.4; P = .014). The colon lengths in this series were 12.8 ± 0.4 versus 10.0 ± 0.3 cm; P < .001). In this experimental series, a further indicator of inflammation, the mean cross-sectional area of the colon wall was assessed with computer-based image analysis in nine colonic sections from each colon (Lehr et al., 1997). The mean area was higher in DSS-exposed mice (2.1 ± 0.2 mm²) compared with mice with normal drinking water (1.3 ± 0.2 mm²; P = .011). In the rolipram-treated DSS-exposed mice, this increased colon wall thickness was completely reversed (1.2 ± 0.1 mm²; P < .001; Fig. 2).

For measurement of colonic TNF concentration, the colon from 15 mice of a third experimental series treated as described above was obtained at day 11 (Fig. 8). Eluate from colonic tissue of DSS-exposed mice without rolipram treatment had the highest TNF concentration of 201 ± 39 pg/mg (wet weight) colonic tissue (n = 4) compared with 115 ± 25 pg/mg colonic tissue (n = 7) in rolipram-treated mice. In the noninflamed colon of control mice receiving rolipram alone, TNF was 97 pg/mg colonic tissue (n = 2) and in mice receiving 0.9% NaCl TNF was 97 pg/mg colonic tissue (n = 2).

Treatment of Established Colitis with Rolipram. To further evaluate the therapeutic value of rolipram, we studied the effect of rolipram on preexisting colitis (delayed treatment protocol). Colitis was induced by the administration of DSS for 7 days. On day 8, after discontinuing DSS administration, rolipram therapy was started. A total of 88 mice was included in two independent series of studies. The first series was designed to assess clinical score and postmortem morphologic parameters at defined time points during resolution of colitis. In the second series, clinical parameters were followed until complete resolution of clinical colitis in treatment and control groups.

The first study included 54 mice, with 7 or 3 mice in each of the four treatment groups available for morphologic examination at the end of the study. The other mice were sacrificed at the indicated time points during the study.

On the first day of treatment (day 8), the groups designated to receive rolipram or 0.9% NaCl, respectively, had
developed similar clinical activity of colitis (2.8 ± 0.2 in the rolipram group; 2.5 ± 0.2 in the NaCl group; N.S.; Fig. 4). After discontinuation of DSS administration, the clinical score in the control group declined gradually until day 15 (1.4 ± 0.3; n = 7). The rolipram-treated mice recovered faster as demonstrated by an earlier decrease and a lower clinical score at day 15 (0.3 ± 0.1; n = 7; P = 0.003).

Changes of the colon length reflected the clinical course (Fig. 5). At the indicated time points, two mice in each group were sacrificed. Beginning at day 10, the colon length in the rolipram group showed an earlier increase (indication of decreased inflammation) compared with the control group. From day 0 to 13, the variation is due to the low number of mice at each time point (two mice in each group). At day 15, all remaining mice were sacrificed (seven mice each in groups with DSS, three mice each in both groups without DSS). The colon length in the rolipram group was significantly longer than in DSS-fed mice given 0.9% NaCl (14.4 ± 0.4 versus 11.9 ± 0.3 cm; P < .001). In the control groups without DSS exposure, the colon length in mice treated with rolipram (17.8 ± 0.2 cm) was unchanged compared with mice injected with 0.9% NaCl (17.5 ± 0.5 cm; data not shown).

On the histologic level, no differences between rolipram-treated mice and the control group were found (histologic score 4.1 ± 1.1 in the rolipram group versus 4.3 ± 0.7 in the control group). For the mean cross-sectional area of the colon, there was only a trend to lower values in the rolipram group (2.8 ± 0.3 mm²) compared with the control group (3.4 ± 0.3 mm²; P = .195). Both were markedly higher than in mice without DSS treatment (1.8 ± 0.1 mm²; P = .010), reflecting inflammation in the DSS-treated mice.

In the second series with the delayed treatment protocol, clinical parameters were followed until complete resolution of clinical signs of colitis. This study included 34 mice, 14 in both DSS-exposed groups and 3 mice in both groups without DSS. After 7 days, the groups designated to receive rolipram or 0.9% NaCl, respectively, had developed the same extent of colitis as in the first series (clinical activity score of the rolipram group, 2.1 ± 0.3, and for the NaCl group, 2.3 ± 0.3; data not shown). Confirming the results of the first series, rolipram-treated mice recovered earlier than control mice. The difference in the clinical activity score between both groups was maximal at day 11 (rolipram group, 1.3 ± 0.3; NaCl group, 2.4 ± 2.1; P = .005) and declined to nonsignificance until day 15 (rolipram group, 0.5 ± 0.2; NaCl group, 0.9 ± 0.3; P = .301). At day 15, the colon length of the rolipram-treated mice was significantly longer (15.2 ± 0.4 cm) than the colon length of untreated mice (13.0 ± 0.3 cm, P < .001; controls without DSS, 17.5 ± 0.3 cm). Differences in the individual scores of body weight, rectal bleeding, and stool consistency between both groups are summarized in Fig. 9B. Rolipram significantly decreased rectal bleeding and the appearance of loose stool. There was a nonsignificant trend to higher body weight in the rolipram group (P = .18).
parallel with DSS did not inhibit or delay onset (~day 4) but decreased aggravation of colitis at later time points (between days 5 and 11; Fig. 7). In accordance with the clinical and histologic results, rolipram suppressed TNF synthesis in the colon of DSS-fed mice. The model of delayed treatment was designed to test the efficacy of rolipram as therapy of preexisting colitis. Rolipram improved recovery of mice from established colitis after discontinuation of DSS treatment. Signs of colitis decreased earlier in rolipram-treated mice than in mice without therapy (Fig. 4).

**DSS Model.** Several experimental models of inflammatory bowel disease have been described (for review, see Elson et al., 1995). The DSS model of colitis has been recommended for preclinical testing of new pharmacologic compounds for therapy of chronic inflammatory bowel disease (Cooper et al., 1993; Elson et al., 1995). DSS-induced colitis has a number of advantages, including its simplicity, the ability to induce both acute and chronic inflammatory lesions, the high degree of uniformity of the lesions, and the reproducibility in respect to both time course and severity among individual mice of a given inbred strain. This uniformity and reproducibility is not achieved in several other experimental models of inflammatory bowel disease (Elson et al., 1995).

**Side Effects of Rolipram.** In general, therapy with rolipram at a dose of 10 mg/kg b.wt./day was well tolerated by the mice. Immediately after injection of rolipram, mice showed reduced motility, but they returned to normal motoric behavior after a few minutes. These changes could not be attributed to the i.p. injection of fluid because changes in the behavior did not occur in control mice injected with 0.9% NaCl. In agreement with previous in vivo studies (Turner et al., 1994; Genain et al., 1995; Gantner et al., 1997; Nyman et al., 1994; Ross et al., 1997) with the same (10 mg/kg b.wt./day) or lower doses of rolipram, we observed no major side effects. For higher doses, side effects in nonhuman primates include vomiting, salivation, and mouth scratching, none of which was observed in the present study (Genain et al., 1995).

Fasting has recently been described to have some protective effect during development of DSS-induced colitis (Savendahl et al., 1997). In our study, in control mice without DSS, injection of rolipram did not influence body weight. In contrast, rolipram-treated DSS mice had higher body weight compared with mice without rolipram. Therefore, it is unlikely that rolipram exerts anti-inflammatory activity by decreasing food intake as a possible mechanism of action.

**Cellular Effects of Rolipram.** The anti-inflammatory activity of rolipram depends on its direct action on leukocytes. We and others have shown that rolipram strongly inhibits TNF production in monocytes and macrophages (Schade and Schudt, 1993; Semmler et al., 1993; Prabhakar et al., 1994; Seldon et al., 1995; Barnette et al., 1996). We have demonstrated that the synthesis of the anti-inflammatory cytokine interleukin (IL)-10 is enhanced by rolipram (Eigler et al., 1998) and that exogenous IL-10 acts synergistically with rolipram in decreasing TNF production (Siegmund et al., 1997). Furthermore, rolipram inhibits IL-2-mediated proliferation of primary T cells but not IL-2 production itself (Essayan et al., 1994) and inhibits γ-interferon synthesis of T cells (Essayan et al., 1994; Sommer et al., 1995).

These anti-inflammatory characteristics of rolipram act in concert to effectively inhibit the inflammatory response in...
vivo (Turner et al., 1994; Genain et al., 1995; Sommer et al., 1995; Gantner et al., 1997).

**Determination of Endpoints in Colitis Model.** We quantified clinical activity with a scoring system that has been described to be a reliable marker of pathologic changes (Cooper et al., 1993). The clinical score was determined in a blinded fashion to exclude bias by the examining person. Shortening of the colon as a morphologic parameter for the degree of inflammation correlates well with pathologic changes (Okayasu et al., 1990). In our studies, the length of the colon proved to be an easily determined and consistent marker of colitis. Histologic examination was performed blinded and included the degree of infiltration by inflammatory cells in the mucosa and the degree of tissue damage. In our study, a histologic score calculated from these two markers was found to parallel clinical changes during induction of colitis.

**TNF-α and Colitis.** There is evidence that TNF plays a central role in inflammatory bowel disease (for review, see van Deventer, 1997; Sandborn and Hanauer, 1999). The therapeutic benefit of TNF inhibition in Crohn’s disease has been shown in clinical studies with chimeric anti-TNF antibodies (van Dullemen et al., 1995; Stack et al., 1997; Targan et al., 1997; Present et al., 1999). Although proving the principle of targeting TNF in inflammatory bowel disease, efficacy of anti-TNF antibody therapy may decrease with time because of the formation of anti-idiotypic antibodies. Furthermore, the development of antinuclear antibodies has been observed with prolonged anti-TNF antibody therapy in patients with rheumatoid arthritis (Elliott et al., 1994).

Anti-TNF antibodies also have been tested in DSS-induced colitis. Neutralization of TNF but not of IL-1 reduced inflammation in the chronic model of DSS-induced colitis in mice (Kojouharoff et al., 1997). In another study, treatment with anti-TNF antibody failed to prevent onset of colitis in the acute model of DSS-induced colitis (Olson et al., 1995). These primarily contradictory results may be due to the protective effect of TNF against bacterial invasion in intact mucosa.

**PDE Inhibition and TNF-α.** Intracellular concentrations of cAMP increase either as a consequence of receptor-triggered adenylyl cyclase activation or by decreased activity of PDEs. Cyclic nucleotide PDEs have been classified into nine distinct families with several subgroups (Beavo, 1995). Beaded adenylyl cyclase activation or by decreased activity of several PDE inhibitors such as rolipram have source of TNF, is PDE IV (Seldon et al., 1995; Souness et al., 1995). Rolipram decreased disease activity (Sommer et al., 1995). In nonhuman primates, rolipram protected against autoimmune demyelinating disease even when administered after sensitization to central nervous system antigens (Genain et al., 1995). In rats, rolipram decreased clinical activity of experimental arthritis (Nyman et al., 1997; Ross et al., 1997). Additionally, LPS-induced TNF synthesis in mice could be suppressed by rolipram (Griswold et al., 1998). Rolipram reduced airway hyper-responsiveness in response to acute and chronic antigen exposure in monkeys (Turner et al., 1994). In this study, antigen-induced increases of TNF but not of IL-1 concentration were inhibited in bronchoalveolar lavage. This is in agreement with our in vitro findings of selective inhibition of TNF but not IL-1 synthesis by rolipram (Semmler et al., 1993).

**Phosphodiesterase Inhibition and Inflammatory Bowel Disease.** To date, specific type IV PDE inhibition has not been tested as a therapeutic strategy for inflammatory bowel disease. However, there are reports on the use of the nonspecific PDE inhibitor pentoxifylline for this indication. In the trinitrobenzene sulfonic acid model of colitis in rats, pentoxifylline treatment reduced the pathologic changes (Peterson and Davey, 1997). Yet, in a small clinical study with 16 patients with corticosteroid-dependent Crohn’s disease, the administration of 400 mg of pentoxifylline administered four times a day did not improve clinical or histologic activity of disease (Bauditz et al., 1997). In vitro studies by the same research group revealed that a high concentration of pentoxifylline ($IC_{50} = 25 \mu g/ml$) was necessary to inhibit TNF synthesis in organ cultures of inflamed mucosa (Reimund et al., 1997). Nonspecific PDE inhibitors are less selective than rolipram and require a 500-fold higher concentration for inhibition of TNF synthesis. In human mononuclear cells, the concentration that inhibits TNF synthesis by 50% ($IC_{50}$) is 70 nM for pentoxifylline and 130 nM for rolipram (Semmler et al., 1993). This limits the therapeutic use of compounds such as pentoxifylline as anti-inflammatory agents.

In the present study, we could demonstrate the suppression of colonic TNF concentrations by rolipram close to TNF concentrations observed in control mice. This emphasizes the mediatory function of TNF in this model.

The present study has some limitations. First, although DSS-induced colitis serves as a model for human disease, the cause of colitis in humans is not known and therefore other pathogenetic mechanisms may be at active. Second, due to the species studied and the particular situation of the animal model, we have examined a short disease course of 11 to 15 days, whereas inflammatory bowel disease in humans is chronic, extending over months and years. And third, i.p. administration is not a practicable route clinically and oral i.v. or topical formulations will have to be tested before use in humans for this indication.

**Acknowledgments**

We thank Dr. Helmut Wachtel, Schering AG, Berlin, for providing rolipram; Prof. Elmar Richter for reviewing the animal study proposal; Prof. Klaus Loeschke, Dr. Ulrich Hacker, Dr. Jochen Moeller, Dr. Christoph Brunner, Katrin Wolf, Anne Krug, Simon Erhardt, Bernd Jahnsoeder, and Uta Emmerich for helpful discussions; and Angela Hackl and Oliver Blank for excellent technical assistance.


