The Specific Type-4 Phosphodiesterase Inhibitor Mesopram Alleviates Experimental Colitis in Mice

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ABSTRACT
Mesopram, a specific inhibitor of type-4 phosphodiesterase, decreases the synthesis of tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). In the present study, we investigated the effect of mesopram in dextran sulfate sodium (DSS)-induced murine colitis. In the preventive model, colitis was induced by DSS simultaneously with the application of mesopram in BALB/c mice. In the therapeutic model, colitis was induced in BALB/c mice by DSS over 7 days. At day 8, DSS was discontinued, and treatment was started. Mesopram was applied intraperitoneally or orally. The clinical score was calculated daily during the course of each study. Post mortem, colon length, histologic score, and expression of TNF-α and IFN-γ in colons were determined. In the preventive model, mesopram significantly reduced the maximal clinical score, decreased colon shortening, and the histologic score. A dose finding study, using the preventive model, showed that most clinical and post mortem benefit was achieved with 50 mg/kg mesopram compared with 2 and 10 mg/kg. In the therapeutic model, i.p. mesopram treatment led to a significant reduction of clinical score. Both, i.p. and p.o. mesopram significantly reversed DSS-induced colon shortening and reduced the ex vivo colonic production of IFN-γ. We conclude that the specific type-4 phosphodiesterase inhibitor mesopram ameliorates murine colitis both in a preventive and a therapeutic setting.

Infiltration of the mucosa by leukocytes is a key feature in the pathogenesis of human inflammatory bowel disease. At inflammatory sites, proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) stimulate endothelial cells to express adhesion molecules on their cell surface (Marui et al., 1993; Speieker et al., 1997; Nakada et al., 1998). These molecules interact with receptors on leukocytes, which subsequently infiltrate from the blood vessels into the mucosa. TNF-α also activates invading T cells and NK cells to produce interferon-γ (IFN-γ). In vitro, IFN-γ increases the sensitivity of colonic epithelial cells to several apoptotic stimuli via up-regulation of caspase-1 (O’Connell et al., 2000). Moreover, IFN-γ exerts indirect cytotoxicity by increasing the release of reactive oxygen species by macrophages. Successful treatment of patients with steroid refractory or fistulizing Crohn’s disease with anti-TNF-α antibody (van Dullemen et al., 1998; Rutgeerts et al., 1999; Sandborn and Hanauer, 1999) demonstrates the anti-inflammatory potency of this specific cytokine blockade (Sandborn and Hanauer, 1999). Repeated administration of anti-TNF-α antibodies, however, may lead to production of anti-chimeric (Sandborn and Hanauer, 1999) or anti-double-strand DNA antibodies (Elliott et al., 1994) or a possible flare up of tuberculosis due to the long-term alteration of the immune defense (Keane et al., 2001). Among the agents known to inhibit the synthesis of TNF-α, attention has focused on cAMP-elevating phosphodiesterase (PDE) inhibitors (Eigler et al., 1997). The predominant PDE isoenzyme in macrophages, the main cellular source of TNF-α, is the type-4 PDE (Gantner et al., 1997). Compared with the nonspecific PDE inhibitor pentoxifylline, the specific type-4 PDE inhibitor rolipram was 500-fold more potent in suppressing TNF-α synthesis in human mononuclear cells (Semmler et al., 1993). In vitro, rolipram not only suppresses the synthesis of proinflammatory cytokines such as TNF-α and IFN-γ but also induces anti-inflammatory cytokines such as interleukin-10 (Eigler et al., 1998). Nevertheless, several adverse effects of rolipram may limit its use in a clinical setting. The most frequent adverse effect reported in clinical trials was nausea (Zeller et al., 1984; Hebenstreit et al., 1989). Moreover, rolipram is a racemic com-

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ABBREVIATIONS: TNF-α, tumor necrosis factor-α; INF-γ, interferon-γ; PDE, phosphodiesterase; DSS, dextran sulfate sodium; HEC, hydroxyethylcellulose; ELISA, enzyme-linked immunosorbent assay.
pound with differing activities and kinetics of the two enantiomers. Thus, it may not meet the state-of-art demands on a new compound. Mesopram is a novel potent and selective enantiomeric PDE4 inhibitor. It has been shown to be effective in the treatment of experimental autoimmune encephalitis in rodents (Dinter et al., 2000).

In our model, colitis was induced by oral administration of dextran sulfate sodium (DSS). DSS-induced colitis is characterized histopathologically by mucosal infiltration of inflammatory cells, focal crypt damage, epithelial injury, and ulceration (Okayasu et al., 1990; Cooper et al., 1993; Dieleman et al., 1997). Like in Crohn’s disease, pathology of DSS-induced colitis exhibits a diffuse alteration of the colon with transmural inflammation and aphthous erosions, although no fissures or granuloma are seen. The pathomechanism of DSS-induced colitis includes both toxic effects on the epithelium and production of proinflammatory cytokines by macrophages, which are stimulated after phagocytosis of DSS. In the present study, we induced colitis in BALB/c mice and investigated the efficacy of the specific type-4 PDE inhibitor mesopram both in the prevention and therapy of colitis. Endpoints were the clinical score, the colon length, the histologic score of the colon, and the local IFN-γ expression.

Additionally a dose finding study of mesopram p.o. was performed using the preventive model with clinical score and colon length as end points. As a reference compound in this trial, olsalazin was used since it was previously shown to be effective in DSS-induced colitis (Zijlstra et al., 1992; Axelsson et al., 1998).

Materials and Methods

Mice. Female, 6- to 8-week old BALB/c mice weighing 20 to 22 g and weighing 16 to 19 g in the dose finding study were used as indicated. The animals were housed in temperature-controlled rooms with a 12-h light/dark cycle. They were fed standard mouse chow pellets, had access to bottled tap water ad libitum, and were acclimatized to the conditions at least 7 days before they were used in experiments. 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 histologic scoring of colitis were performed in a blinded experimental design.

Reagents. Brefeldin A, phorbol myristyl acetate, and ionomycin were purchased from Sigma-Aldrich (Munich, Germany), RPMI 1640 medium from Biochrom (Berlin, Germany), and fetal calf serum from Invitrogen (Karlsruhe, Germany). Mesopram was kindly supplied by Schering AG (Berlin, Germany). It was stored and dissolved as indicated in the data sheet. Olsalazine (Dipentum) was purchased from Pharmacia and Upjohn (Erlangen, Germany).

Induction and Treatment of Colitis. Mice were fed 3.5% DSS (lot no. 4470 C; molecular mass 30–40 kDa; ICN, Eschwege, Germany) dissolved in sterile, distilled water ad libitum at days 1 to 11 in the preventive model or days 1 to 7 in the therapeutic model. Mesopram (10 mg/kg b.wt. q.d.) was administered p.o. or i.p. at days 1 to 10 in the preventive model or at days 8 to 14 in the therapeutic model. In the dose finding study, mesopram was administered orally in doses of 2, 10, or 50 mg/kg b.wt. q.d.. When applied orally, mesopram was suspended in a solution of 0.5% hydroxyethylcellulose (HEC; lot no. S29089015; Schuchard, Germany) as vehicle. When administered intraperitoneally, as done in the therapeutic model, mesopram was dissolved in a solution of 10% Cremophor EL (BASF, Ludwigshafen, Germany) as vehicle and filtered through syringe filters (0.2 µm; Gelman Sciences, Ann Arbor, MI). Placebo-treated animals received oral vehicle without mesopram. Control mice were given bottled tap water without DSS ad libitum and received mesopram (10 and 50 mg/kg b.wt. p.o. q.d.) or vehicle p.o. q.d.. The reference compound olsalazine, used in the dose finding study, was administered orally in a dose of 40 mg/kg b.wt. dissolved in 0.5% HEC. The volume of application was 200 µl both orally and intraperitoneally.

Evaluation of Clinical Score, Colon Length, and Histologic Score. Body weight, fecal blood, and stool consistency were determined daily (Cooper et al., 1993; Hartmann et al., 2000; Siegmund et al., 2001a). Two investigators blinded to the treatment groups assessed the clinical score as indicated in Table 1. The average of these three scores (body weight, stool consistency, and fecal blood) gave an overall clinical score ranging from 0 (healthy) to 4 (maximal activity of colitis).

Post mortem, the entire colon was removed from the caecum 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 histologic analysis. Sections were stained with H&E. Histologic scoring was performed in a blinded manner by a pathologist (Hartmann et al., 2000; Siegmund et al., 2001a). 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 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 histologic score ranged from 0 (no changes) to 6 (extensive cell infiltration and tissue damage).

All live animals of the treatment groups were included in the scores evaluated. Mice that died during the treatment period in the preventive and treatment model were scored as maximally ill, as they showed high clinical activity scores the day before they died. Concerning the clinical score, these animals were counted as score 4 (equals maximally ill) on the day of death. Nevertheless, these animals had to be excluded from the evaluation of post mortem and ex vivo parameters. As the definite cause of death in the dose finding study was not always clearly related to colitis, all mice that died before the end of study were excluded from statistical analysis.

Colon Cytokine Synthesis. To study colon homogenate at the end of the experimental course, colonos were removed, and strips of the colon (about 1 cm) were mechanically crushed, vigorously vor-

### TABLE 1

<table>
<thead>
<tr>
<th>Score Points</th>
<th>Weight Loss %</th>
<th>Stool Consistency</th>
<th>Rectal Bleeding</th>
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<tbody>
<tr>
<td>0</td>
<td>&lt;0</td>
<td>Formed</td>
<td>Negative hemoccult</td>
</tr>
<tr>
<td>1</td>
<td>0–4.9</td>
<td>Pasty, formed</td>
<td>Positive hemoccult</td>
</tr>
<tr>
<td>2</td>
<td>5–9.9</td>
<td>Pasty to soft, uniformed</td>
<td>Traces of blood on stool</td>
</tr>
<tr>
<td>3</td>
<td>10–20</td>
<td>Diarrhea</td>
<td>Gross macroscopic bleeding</td>
</tr>
<tr>
<td>4</td>
<td>&gt;20</td>
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toxid for 1 min in 200 µl of tissue protein extraction reagent (Pierce Chemical, Rockford, IL) and shock frozen in liquid nitrogen, as previously described (Hartmann et al., 2000; Siegmund et al., 2001a). The homogenate was centrifuged at 10,000g at 4°C for 15 min. The amount of total extracted protein was determined by Bradford analysis using BioRad Protein Assay (BioRad Laboratories, Munich, Germany) as the dye reagent.

To study ex vivo cytokine synthesis, strips of colon (about 1 cm) were thoroughly washed in a solution of 100 U/ml penicillin and 100 mg/ml streptomycin in phosphate-buffered saline (Roche Diagnostics, Ingelheim, Germany), weighed, and incubated in RPMI 1640 medium (Biochrom, Berlin, Germany) containing 10% fetal calf serum at 37°C in a humidified atmosphere with 5% CO2 over a period of 20 h, as described by Siegmund et al., (2001b). To keep the conditions as close as possible to the in vivo situation, no cell stimulant (like phorbol myristyl acetate) was added. To obtain combined lysate plus supernatant, the samples received three freeze-thaw cycles before determining the total cytokine concentration. The amount of TNF-α and IFN-γ in the colon homogenate or in the combined lysate plus supernatant of colon culture was quantified by ELISA (BD Biosciences, Heidelberg, Germany) according to the manufacturer’s instructions and adapted to the weight of the tissue probe.

Statistical Analysis. Data are expressed as means ± S.E.M. Statistical significance 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

Preventive Model and Dose Finding Study

Clinical Score. Mice fed with 3.5% DSS developed signs of colitis at day 5, defined by a clinical score >0.5. Oral treatment with 10 mg/kg q.d. mesopram delayed the onset of colitis for 1 day and reduced the progression of the disease until the end of the study (Fig. 1A). At day 10, placebo-treated animals suffered from a severe colitis, with a clinical score of 3.2 ± 0.2 (n = 8), whereas colitis was significantly blunted in the 10 mg/kg q.d. mesopram-treated group (clinical score 1.8 ± 0.3; n = 7; p = 0.004). Each individual parameter of the clinical score was significantly improved in the mesopram-treated group. The score of weight loss was 3.3 ± 0.3 in the placebo group versus 2.3 ± 0.3 in the mesopram group (p = 0.041), the stool consistency score was 2.8 ± 0.4 versus 1.0 ± 0.5 (p = 0.017), and the fecal blood score was 3.5 ± 0.3 versus 2.0 ± 0.4 (p = 0.010). None of the control animals, which were not exposed to DSS and received either mesopram (10 mg/kg b.wt. q.d.; n = 4) or vehicle HEC (n = 4), showed signs of colitis throughout the experimental course.

In the dose finding study, mesopram dose dependently decreased the severity of colitis (Table 2). Mesopram (50 and 10 mg/kg) significantly improved the clinical score at day 10 compared with the placebo-treated group (clinical score 1.5 ± 0.6 and 2.4 ± 0.6, respectively, versus 3.3 ± 0.6; p = 0.0067, p < 0.026). The individual parameters, stool consistency and fecal blood, improved significantly in those two groups; the score of weight loss showed no significant difference. Mesopram in a dose of 2 mg/kg b.wt. showed a tendency toward improving the clinical score, but this effect was not significantly different. Olsalazine as a reference compound showed no significant improvement of the clinical score. For all described data, see Table 2 and Fig. 1B.

Colon Length. DSS led to a reduction of mean colon length in the placebo-treated group (8.6 ± 0.4 cm, n = 8) compared with the nonexposed control groups (Fig. 2A) (14.3 ± 0.2 cm, n = 4, in the HEC-treated control group; 14.5 ± 0.3 cm; n = 4, in the 10 mg/kg q.d. mesopram-treated control group). Oral treatment with 10 mg/kg mesopram significantly (p < 0.001) reduced the extent of DSS-induced colon shortening to 11.5 ± 0.2 cm (n = 7).

In the dose finding study, mesopram showed a dose-dependent effect on colon length shortening (Fig. 2B) with 9.3 ± 2.2 cm in the mesopram 50 mg/kg b.wt. group compared with 6.1 ± 0.7 cm (p < 0.03) in the placebo-treated group. Mesopram (10 mg/kg) lead to a colon length of 8.3 ± 1.0 cm (p < 0.001) and 2 mg/kg mesopram to a colon length of 6.9 ± 1.1 cm (not significant). Olsalazine had no therapeutic effect with a colon length of 6.5 ± 0.4 cm. Both control groups (no DSS and either HEC or mesopram 50 mg/kg) showed a colon length of 10.8 ± 1.6 and 12.0 ± 1.7 cm, respectively (p < 0.0001 compared with the placebo-treated group).

Histologic Score. The mucosal damage caused by DSS was quantified by a pathologist in a blinded manner. Colonis of DSS-exposed mice revealed mucosal erosions and crypts of villi.
destruction as well as an inflammatory infiltrate composed of macrophages, lymphocytes, eosinophils, and a few neutrophils. In DSS-exposed groups, oral treatment with mesopram reduced the mean histologic score (0 for no changes, 6 for maximal tissue damage and cell infiltration) from 4.2 ± 0.3 in the HEC-treated group (n = 7) to 2.9 ± 0.3 in the mesopram-treated group (n = 7). This effect was statistically significant (p = 0.003) (Fig. 3). Neither control group showed any histologic sign of colonic inflammation evidenced by a score of 0.3 ± 0.1 in the HEC-treated group (n = 4) and 0.4 ± 0.2 in the mesopram-treated group (n = 4).

Colonic Content of Proinflammatory Cytokines. For measurement of the colonic content of the proinflammatory cytokines TNF-α and IFN-γ, colon samples were homogenized at day 11. TNF-α and IFN-γ were quantified in the homogenate by ELISA. Non-DSS-exposed groups showed the lowest level of colonic TNF-α (17 ± 3 pg/mg of protein, n = 4 in the HEC-treated control group; and 17 ± 1 pg/mg of protein, n = 4 in the mesopram-treated control group). DSS treatment led to an increased content of TNF-α in the colon (41 ± 10 pg/mg of protein, n = 7) (Fig. 4A). Although not significantly different, an oral treatment condition with 10 mg/kg mesopram markedly reduced TNF-α (25 ± 6 pg/mg of protein, n = 7) compared with the placebo-treated group.

The content of IFN-γ (Fig. 4B), another key proinflammatory cytokine that is induced by TNF-α, was also reduced in the colon tissue of mesopram-treated, DSS-exposed animals (61 ± 13 pg of IFN-γ/mg of protein, n = 7) compared with the placebo-treated DSS-exposed group (76 ± 17 pg of IFN-γ/mg of protein, n = 7). Consistent with the findings for TNF-α, the lowest content of IFN-γ was detected in the colons of the non-DSS-exposed control groups (56 ± 7 pg of IFN-γ/mg of protein, n = 4 in the mesopram-treated control group and 51 ± 11 pg of IFN-γ/mg of protein, n = 4 in the placebo-
Mesopram Improves Experimental Murine Colitis

Treatment of Established Colitis in BALB/c Mice

Clinical Score. The efficacy of mesopram was also investigated in the treatment of pre-existing colitis. Colitis was induced in BALB/c mice by feeding them 3.5% DSS dissolved in the drinking water ad libitum over a period of 7 days. After replacing DSS by normal drinking water at day 8, DSS-exposed mice were stratified into three groups according to their clinical score, each group containing eight equally ill mice. Two control groups containing four mice did never receive DSS. Starting at day 8, these groups were treated with 10 mg/kg q.d. mesopram i.p. (dissolved in the vector 10% Cremophor EL and ultra-filtered) or p.o. (dissolved in 0.5% HEC solution) or placebo (0.5% HEC solution p.o.). The two control groups received from day 8 mesopram (10 mg/kg b.wt. orally q.d.) or the vehicle (0.5% HEC). At day 7, the clinical score of all DSS-exposed groups was significantly ($p < 0.05$) higher than in control mice, which showed no signs of colitis (Fig. 5). Although DSS was discontinued at day 8, all DSS-exposed groups initially showed a progression of the clinical signs of colitis, which reached its maximum at day 9 in all groups. The extent of colitis was constant in the placebo-treated group at days 9 through 12. At day 15, the clinical score of this group ($n = 8$) had improved from a maximum of $2.1 \pm 0.1$ to $1.2 \pm 0.2$ reflecting the spontaneous course of the disease. Mice that received mesopram orally ($n = 8$) had the most severe colitis at day 9 compared with the other groups ($3.0 \pm 0.3$ versus $2.1 \pm 0.1$ in the placebo group and $2.0 \pm 0.2$ in the intraperitoneally treated group). At day 15, the intraperitoneally mesopram-treated group ($n = 8$) had recovered from colitis with a clinical score of $0.4 \pm 0.2$. The clinical score was significantly lower compared with the placebo-treated group ($1.2 \pm 0.2$, $p = 0.020$).

Colon Length and Histology. Treatment with DSS leads to a shortening of the colon as a post mortem marker of the extent of colitis (Fig. 6). DSS-exposed, placebo-treated mice ($n = 8$) showed significantly reduced colon lengths compared with mice that were not exposed to DSS ($10.3 \pm 0.3$ versus $14.8 \pm 0.7$ cm, $n = 4$ in mesopram-treated control mice; and $14.5 \pm 0.5$ cm, $n = 4$ in placebo-treated control mice, $p < 0.001$). Mesopram treatment of established colitis over a period of 7 days reversed the DSS-induced colon shortening compared with placebo-treated, DSS-exposed mice ($12.0 \pm 0.2$ cm, $p < 0.001$, $n = 8$ in the i.p. group; and $12.2 \pm 0.2$ cm, $p < 0.001$, $n = 8$ in the p.o. group). Histologic analysis of the colons confirmed the absence of inflammation in both non-DSS-exposed control groups (Fig. 7). In contrast, all DSS-exposed groups showed a histologic score that reflected colitis, which was induced in these animals during the 1st week of the experimental course. Intraperitoneally mesopram-treated mice showed a trend toward an improved histologic score ($2.8 \pm 0.2$, $n = 8$), whereas orally mesopram-treated mice ($3.3 \pm 0.4$, $n = 8$) did not differ from placebo-treated mice ($3.6 \pm 0.3$, $n = 8$) in their histologic score.

IFN-γ Synthesis by Colonic Tissue. To evaluate the ex vivo production of IFN-γ by colonic tissue, colons were removed.
moved at day 15. Strips of the colon of about 1 cm in length
were prepared and incubated overnight, as indicated in the
method section. After an incubation period of 18 h, the con-
centration of IFN-γ/H9253 in the combined lysate plus conditioned
medium was quantified by ELISA and adapted to the weight
of the tissue (Fig. 8). The highest amounts of IFN-γ were
detected in the combined lysate plus conditioned medium of
colons obtained from DSS-exposed, placebo-treated mice (121
ng of IFN-γ/H9253/100 mg of colon, n/H11005 8). Both, intraperitoneal
and oral treatment with mesopram reversed significantly the DSS-
induced shortening of the colon. Values are depicted as means ± S.E.M.

Fig. 6. Mesopram partially reverses DSS-in-
duced colon shortening in established colitis.
Mice were fed 3.5% DSS for 7 days. At day 8,
DSS was discontinued, and mice received ei-
ther mesopram (10 mg/kg i.p. or p.o.) or pla-
cebo. At day 15, colon length was measured.
DSS induced a significant reduction of colon length compared with control animals. Both, intraperitoneal and oral treatment with me-
sofram reversed significantly the DSS-
induced shortening of the colon. Bars represent
means ± S.E.M.

Fig. 7. Histologic analysis of colons with established DSS-induced colitis.
Mice were fed 3.5% DSS for 7 days. At day 8, DSS was discontinued and
mice received either mesopram (10 mg/kg i.p. or p.o.) or placebo. At day
15, histologic score (0 for no inflammation, 6 for maximal tissue damage,
and cell infiltration) was determined in a blinded manner. Exposure to
DSS led to an increase of the histologic score compared with control mice.
There was a trend toward an improvement of the histologic score in the i.p.
mesopram-treated group. Values are depicted as means ± S.E.M.

Discussion
In the present study, we demonstrate the in vivo efficacy of
the specific type-4 phosphodiesterase inhibitor mesopram in
the treatment of established DSS-induced murine colitis and
its efficacy in the prevention of DSS-induced colitis with two
different routes of administration. In an additional dose find-
ing study of orally administered mesopram (2, 10, and 50
mg/kg b.wt. q.d.), mesopram dose dependently ameliorated
DSS-induced colitis with significant improvement in the clin-
ical score and colon length.

Oral treatment with mesopram effectively prevented DSS-
induced colitis in BALB/c mice. In concordance with its clin-
ical efficacy, oral mesopram reduced colon shortening as a
marker of colitis. Furthermore, oral administration of meso-
pram partially prevented histologic signs of colitis, and col-
onic specimens obtained from mesopram-treated animals
showed a trend toward a reduced content of the proinflam-
atory cytokines TNF-α and IFN-γ. In the therapeutic
model, both intraperitoneal and oral mesopram led to an
increased recovery from established colitis. Animals treated
intraperitoneally with mesopram even recovered completely
from colitis within the 7-day treatment period. The in vivo
efficacy was paralleled by a partially reversed colon shorten-
ing and by a reduction of colon IFN-γ synthesis to the level
of non-DSS-exposed control mouse length in both mesopram
groups. In contrast, colons obtained from placebo-treated
mice produced a significantly higher amount of IFN-γ.

The DSS model of murine colitis has shown to be useful for
preclinical testing of new compounds for therapy of human

Fig. 8. Mesopram reduced the ex vivo production of IFN-γ in colonic
tissue in the treatment model of DDS-induced colitis. At day 15, strips of
colons were incubated without stimulus overnight. The content of IFN-γ
in these conditioned medium plus cell lysate was determined by ELISA.
Both p.o. and i.p. treatment of mice with mesopram in vivo significantly
reduced the ex vivo production of IFN-γ by cultured colonic tissue com-
pared with placebo. Values are depicted as means ± S.E.M.
inflammatory bowel disease (Cooper et al., 1993; Elson et al., 1995). Therapeutic agents that are in clinical use or are evaluated in clinical trials, such as olsalazine (Zigelstra et al., 1992; Axelsson et al., 1998), anti-TNF-α antibodies (Murthy et al., 1999), or interleukin-10 (Tomoyose et al., 1998) have been successfully tested in this model. The advantages of this model include its simplicity and the high degree of uniformity and reproducibility of the colonic lesions (Elson et al., 1995). The principle endpoint of this study was the clinical disease activity, which was scored with a system that has been described to correlate with the pathologic changes (Cooper et al., 1993; Hartmann et al., 2000; Siegmund et al., 2001a). As a reference compound olsalazine (40 mg/kg b.wt.) was applied. Surprisingly, and in contrast to the study by Axelsson et al. (1998) orally administered olsalazine in our study did not show a significant improvement in either clinical score or colon length. This underlines the potency of phosphodiesterase type-4 inhibitors in this experimental model.

Post mortem, two further endpoints were evaluated: 1) the shortening of the colon as a morphometric surrogate parameter for the degree of inflammation, which correlates with the pathologic changes and has proved to be a consistent marker of colitis (Okayasu et al., 1990; Hartmann et al., 2000; Siegmund et al., 2001a) and 2) histologic assessment of the tissue damage and extent of infiltration by inflammatory cells.

TNF-α and IFN-γ are both key cytokines in human Crohn’s disease. The local production of these proinflammatory cytokines in murine colonic tissue was quantified ex vivo by ELISA. "In additional experiments (10 mg/kg mesopram i.p. q.d.) with the TH1-biased mouse strain C57BL/6J we could furthermore..."
demonstrate a trend toward a suppressed synthesis of TNF-α and IFN-γ in splenocytes by fluorescence-activated cell sorting analysis (data not shown). This hints at a systemic effect of mesopram on the inflammatory response. These results are consistent with the suppression of IFN-γ synthesis in peripheral blood mononuclear cells of patients with multiple sclerosis or atopic dermatitis by the specific type-4 phosphodiesterase inhibitor rolipram (Ostlere et al., 1997; Navikas et al., 1998).

Preclinical research and first clinical trials provide evidence that specific inhibitors of type-4 phosphodiesterase may represent a new class of anti-inflammatory drugs to treat different kinds of chronic inflammatory diseases. Since cAMP additionally mediates relaxation of bronchial smooth muscle cells, airway diseases such as asthma and chronic obstructive pulmonary disease have been selected as the first target indications (Doherty, 2000). Results from clinical trials with the specific type-4 phosphodiesterase inhibitor air flow in patients with chronic obstructive pulmonary disease are positive (Hay, 2000; Compton et al., 2001). A recent study reports on the successful inhibition of experimental autoimmune encephalitis in rodents by mesopram (Dinter et al., 2000; Francischi et al., 2000; Hofinger et al., 2001), and, as demonstrated here, chronic inflammatory bowel diseases.

We conclude, that specific inhibition of type-4 phosphodiesterase by mesopram may form a novel attractive therapeutic strategy in the treatment of chronic inflammatory diseases and warrants to be evaluated in a clinical trial.

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References


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