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Effects of tumour necrosis factor-α synthesis inhibitors on rat trinitrobenzene sulphonic acid-induced chronic colitis

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Abstract

The fact that tumour necrosis factor-α (TNF-α) is clearly involved in the pathogenesis of intestinal bowel disease, especially Crohn's disease, suggests that TNF-α synthesis inhibitors could be beneficial for treatment. The present study assessed the effect of chronic oral gavage of two in vitro TNF-α synthesis inhibitors, JM 34 maleate or [*N*-(4,6-dimethylpyridin-2-yl)-furane-2-carboxamide)] maleate and XC 21 or (*N*-βpicolyl-tetrafluorophtalimide), on colonic inflammation in trinitrobenzene sulphonic acid-induced colitis in rats. Rats received JM 34 maleate (100 mg/kg) and XC 21 (50 mg/kg) 1 h before colitis induction and then daily for 8 days by oral gavage. The colon was removed on day 8 and processed for clinical score, myeloperoxidase activity, and soluble TNF-α release. Treatment with XC 21, as well as dexamethasone and sulphasalazine, reduced colonic damage and decreased (except with dexamethasone) the incidence of diarrhoea. JM 34 maleate failed to improve the clinical signs of chronic colitis. After trinitrobenzene sulphonic acid-induced colitis, myeloperoxidase activity and TNF-α colonic mucosal production were substantially increased compared to the control (saline instillation). Both of these inflammatory indicators were then significantly decreased ($P \leq 0.05$) after the four chronic treatments (JM 34 maleate, XC 21, sulphasalazine, and dexamethasone). XC 21 appeared to be as efficient as sulphasalazine in improving colonic inflammation. © 2001 Published by Elsevier Science B.V.

Keywords: TNF-α (Tumour necrosis factor-α); Colitis, rat; Inflammatory bowel disease; Thalidomide derivative; Phthalimide derivative; Heterocarboxamide; JM 34 maleate

1. Introduction

Tumour necrosis factor-α (TNF-α) is a key cytokine synthetised by different types of cells (e.g. macrophages and mast cells) during the first stage of the inflammatory process. In association with interleukin-1β, this most pivotal pro-inflammatory cytokine induces enzyme activities (nitric oxide synthase, phospholipase A_2, cyclo-oxygenases, proteases) that stimulate adhesion molecules and the production of cytokines such as interleukin-2, interleukin-10, and interferons. As different chemical structures have been described (Black et al., 1997; Eigler et al., 1997), TNF-α inhibitors could act through different pathways: inhibition of TNF-α synthesis and production, inhibition of TNF-α release (metalloproteases), or inhibition of TNF-α biological activity (soluble receptors). TNF-α production inhibitors have different targets: pre-transcriptional phosphodiesterase IV [rolipram, pentoxifylline, or SB-207499 (Teixeira et al., 1997)] or mitogen-activating protein (MAP) kinase [p38 inhibitors (Badger et al., 1996; Akerlund et al., 1999)].

A new heterocarboxamide, JM 34 maleate, previously synthetised and studied in our laboratories (Robert et al., 1995), inhibits TNF-α production in vitro on lipopolysaccharide-stimulated macrophages. It appears to act mainly at a post-transcriptional level as well as pre-transcriptionally through partial inhibition of extracellular-related kinase-2 (ERK-2) phosphorylation (Vernhet et al., 1997). However, it has recently been determined that thalidomide inhibits TNF-α production by decreasing the half-life of TNF-α mRNA (Moreira et al., 1993). This prompted us to study a new tetrafluorophthalimide derivative (Collin et al., 1999) and the structurally related heterocarboxamide in an in vivo model of inflammation.

Intestinal bowel disease, especially Crohn's disease, is characterised by transmural infiltration and mucosal disruption, in which TNF-α production has recently been

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implicated (Guimbaud et al., 1998; Schreiber et al., 1999). Unfortunately, Crohn's disease is not a single clinical entity, so that the exact aetiology remains unknown. A disturbed balance has been found between pro-inflammatory and anti-inflammatory cytokines (Rogler and Andus, 1998; Schreiber, 1998). Current therapy for intestinal bowel disease includes salicylate derivatives (5-aminosalicylic acid and sulphasalazine) and glucocorticosteroids, but also immunosuppressors and immunomodulators such as azathioprine and methotrexate (Rutgeerts and Vermeire, 1996). Promising results have been obtained with anti-TNF-α therapy in acute and chronic lesions in animal models (Videla et al., 1998) and in human clinical trials using chimeric anti-TNF-α antibodies in refractory Crohn's disease (D'Haens et al., 1999). However, the major disadvantages of these antibodies are adverse immune effects, the need for parenteral administration (Bickston and Cominelli, 1998), and high cost. In this context, the strategy of using TNF-α synthesis inhibitors to treat Crohn's disease would appear to be an interesting alternative.

The purpose of the present study was to assess the efficacy of chronic oral administration of two in vitro TNF-α synthesis inhibitors (JM 34 maleate and XC 21: a heterocarboxamide and a phthalimide derivative, respectively) in an in vivo experimental trinitrobenzene sulphonic acid-induced colitis model in rats. In a first experiment, inflamed colonic wall samples were cultured to quantify TNF-α production 5 days after induction and to assess potential inhibition ex vivo obtained with TNF-α synthesis inhibitors. In a second experiment, the effect of the two compounds on trinitrobenzene sulphonic acid-induced colitis was assessed after daily treatment for 8 days in terms of clinical and biochemical parameters (mucosal TNF-α production and myeloperoxidase activity). The efficacy of the two compounds was compared with that of reference drugs used in the treatment of Crohn's disease (dexamethasone and sulphasalazine).

2. Materials and methods

2.1. Animals

Male Wistar rats (AF) weighing 220 to 260 g were obtained from CER Janvier (France) and housed in cages (a maximum of three animals per cage). Feeding was discontinued 24 h before colitis induction, and the animals were then given water and food ad libitum during the experiments. The care and use of the rats were in accordance with French guidelines for experiments with laboratory animals (Law No. 87-848).

2.2. Inflammation induction

Colitis was induced according to the procedure described by Morris et al. (1989). Briefly, fasted rats were slightly anaesthetised with pentobarbital (30 mg/kg), and colitis was induced 15 min later by administration of 1 ml trinitrobenzene sulphonic acid solution (30 mg solubilised in 40% ethanol) into the lumen of the colon, using a tuberculin syringe fitted with 8-cm gastric intubation.

The animals were maintained in a head-down position for 30 min to prevent leakage of the intracolonic instillate. They were killed 5 or 8 days later after trinitrobenzene sulphonic acid administration. The distal 10-cm portion of the colon was removed and cut longitudinally, slightly cleaned in sterile water to remove faecal residues, and weighed. Different control groups were created, using instillation of ethanol 40% (Lamrani et al., 1999) or saline instillation, for comparison with TNBS/ethanol instillation. According to biochemical parameters and macroscopic damage scores, no inflammation and no difference were observed between the two control groups at day 8.

2.3. Drug administration

Rats were checked daily for behaviour, body weight, and stool consistency.

In a first set of experiments, TNF-α production was assessed 5 days after trinitrobenzene sulphonic acid instillation. In two independent experiments with 12 and 10 rats per group, the effect of four compounds on ex vivo soluble TNF-α production was evaluated. The different dose ranges of dexamethasone (0.01–0.1–1–10 μM), JM 34 maleate (50–100–200–300 μM), XC 21 (1–10–50 μM), and sulphasalazine (50–100–200 μM) were added to RPMI 1640 (containing 10% foetal calf serum) just before the addition of inflamed colon tissue in order to evaluate their potential inhibitory effect on TNF-α production ex vivo. The compounds studied were dissolved in saline or dimethylsulphoxide (final concentration not exceeding 0.2%). Distal colon was removed and carefully rinsed with sterile saline and RPMI 1640. A piece of distal colon weighing about 50 mg was then cultured for 5 h, as described below.

In a second set of experiments, drug effects were assessed in the four treatment groups 8 days after intracolonic instillation of trinitrobenzene sulphonic acid (the control group received NaCl). The four trinitrobenzene sulphonic acid groups were treated once daily for 8 days by oral gavage, with the first administration 1 h before trinitrobenzene sulphonic acid. JM 34 maleate (100 mg/kg), XC 21 (50 mg/kg), dexamethasone (1 mg/kg), and sulphasalazine (100 mg/kg) were dissolved or suspended in methylcellulose 1%. No control groups were used for intracolonic saline instillation or for JM 34 maleate , XC 21, dexamethasone and sulphasalazine. Several tests, such as carrageenan-induced rat paw oedema and PMA-induced mouse ear-swelling, were performed with these dose ranges, and no direct unwanted effects of these compounds on the colon were observed (Collin et al., 1999; Lang et al., 1995). A total of 57 rats were included in the protocol:

9 for each of the four treatment groups plus the control group, and 12 untreated animals.

2.4. Assessment of inflammation

After rats were sacrificed, colonic damage was assessed macroscopically. For each animal, macroscopic inflammation scores were assigned based on the clinical features of the colon, the presence of adherences, and/or stool consistency (Table 1). Pieces of inflamed colon were collected and snap-frozen in liquid nitrogen to quantify myeloperoxidase activity. TNF-α production was evaluated on fresh inflamed tissue.

2.5. Myeloperoxidase activity

Myeloperoxidase is a common constituent of neutrophils, and its activity can be regarded as an index for the severity of digestive inflammation (Krawitz et al., 1984).

About 50 to 100 mg of fresh tissue were suspended in freshly prepared 0.5% hexadecyl trimethylammonium bromide in 50 mM phosphate buffer (pH 6, 1 ml) and immediately stored at −80 °C after necropsy for no longer than 7 days (Ribbons et al., 1997). Tissues were homogenised three times at 4 °C with a Polytron (Bioblock Scientific, France) at 15,000 rpm for 30 s. Homogenates were immediately frozen at −80 °C and then thawed at 37 °C. This freeze-thawing cycle was repeated twice. Samples were then centrifuged at 16,000 rpm for 15 min at 4 °C, and 100 μl of supernatant were added to 2.88 ml of *O*-dianisidine in phosphate buffer solution (0.167 mg/ml, pH 6) containing hydrogen peroxide (0.3%, 20 μl). Absorbance at 470 nm was measured for 2 min using a Kontron Uvikon 960 spectrometer. One unit of myeloperoxidase activity is able to convert 1 μmol of hydrogen peroxide to water in 1 min at 25 °C. Myeloperoxidase activity was expressed as mIU/mg protein content. Protein content was determined using the bicinchoninic acid solution (BCA) protein assay.

Table 1
Criteria for scoring macroscopic damage to trinitrobenzene sulphonic acid-instilled rat colon

Score	Criteria
Ulceration	
0	No damage
1	Focal hyperemia
2	Ulceration without hyperemia or bowel wall thickening
3	Ulceration with inflammation at 1 site
4	= or > 2 sites of ulceration and inflammation
5	Major sites of inflammation > 1 cm along the organ
6–10	Major sites of inflammation > 2 cm along the organ
Adhesions	
0	No adhesion
1	Minor adhesion
2	Major adhesion
Diarrhoea	
0	Absence
1	Presence

2.6. Culture ex vivo

After fresh inflammatory tissues were cut out and weighed, TNF-α production from each colon was quantified in 24-well plates in RPMI 1640 containing foetal calf serum 10% supplemented with glutamine/penicillin/streptomycin/gentamicin (2 mM, 50 IU, 50 mg/ml, and 20 mg/ml, respectively) for 5 h. Supernatants were collected and centrifuged (12,000 rpm for 15 min at 4 °C) to remove bacterial contamination.

2.7. Production of soluble tumour necrosis factor-α

Aliquots of the resulting supernatants were evaluated for TNF-α content using a WEHI 164 clone 13 cytotoxic model assay (Espevik and Nissen-Meyer, 1986; Lang et al., 1995). Briefly, 50 μl of supernatant were added to 50 μl of actinomycin D-treated (2 mg/ml) WEHI cells (6×10^5 cells/ml) in flat-bottomed 96-well plates and incubated for 18 h at 37 °C, 5% CO_2. In each experiment, a reference curve was obtained using serial dilutions of mouse recombinant TNF-α (Boehringer Mannheim, Meylan, France), from 100 pg/ml down to 0.02 pg/ml. After incubation, 50 μl of tetrazolium salts (2.5 mg/ml in phosphate buffer solution) were added to each well and incubated for 3 h. Formazan crystals were solubilised with 100 μl of lysis buffer (1V dimethylformamide, 2V sodium dodecyl sulphate 30%, adjusted to pH 4.7 with acetic acid), and absorbance was measured at 570 nm with an enzyme-linked immunosorbent assay plate reader (Molecular Devices, San Francisco, CA). Assay sensitivity was lower than 1 pg/ml. TNF-α production was expressed as ng/g of wet tissue.

2.8. Drugs

2,4,6-Trinitrobenzene sulphonic acid aqueous solution (5%), dexamethasone, sulphasalazine, and the reagents for the myeloperoxidase assay were obtained from Sigma (Sigma Chimie, St. Quentin Fallavier, France). JM 34 maleate or *N*-(4,6-dimethylpyridin-2-yl)-furane-2-carboxamide maleate and XC 21 or (*N*-βpicolyl-tetrafluorophtalimide) were synthesised in the Therapeutic Chemistry Laboratory (Prof. Le Baut, Nantes, France: Robert et al., 1995; Collin et al., 1999). Methylcellulose and the BCA kit for protein assay were provided by Colorcon (Orping-

ton, England) and Pierce (Interchim, Montluçon, France), respectively.

2.9. Statistical analysis

All data are expressed as the mean ± standard error of the mean (S.E.M.) for *n* rats per experimental group. Myeloperoxidase activity and TNF-α assays were done in triplicate. Statistical analysis was performed with SPSS 10.0 Software. Results for treatment effects on myeloperoxidase activity were studied by one-way analysis of variance (ANOVA), using Tukey's method as post hoc test. The effect of chronic treatment on TNF-α production and clinical scores was analysed using the Kruskall–Wallis test, with the Mann–Whitney *U*-test as post hoc test. A P value < 0.05 was considered as significant.

3. Results

3.1. Colonic inflammation induced by trinitrobenzene sulphonic acid instillation

Compared with the control group, trinitrobenzene sulphonic acid/ethanol intracolonic instillation in experimental groups caused severe diarrhoea in conjunction with rectal bleeding until day 5. Rats showed prostration, piloerection and hypomotility. These symptoms were apparent until day 3. Five days after trinitrobenzene sulphonic acid administration, body weight loss was 5.3 ± 3.6 g, whereas the control group gained 32 ± 2.8 g per animal (Table 2). After 5 days, the weight of the trinitrobenzene sulphonic acid group increased. After saline instillation, no damage was observed macroscopically on the distal colon, myeloperoxidase activity was absent, and TNF-α production was very low (less than 0.1 ng/g of fresh tissue) (Table 2). Rats treated with trinitrobenzene sulphonic acid/ethanol instillation showed severe colonic mucosal damage, with oedema, deep ulcerations and haemorrhage.

Table 2
Parameters quantified 5 days after trinitrobenzene sulphonic acid/ethanol colitis induction in rats

	Control	TNBS
Increase in body weight (g)	32.0 ± 2.8	−5.3 ± 3.6[a]
Macroscopic damage score	0	7.7 ± 1.2[a]
MPO activity mUI/mg (protein)	nd[b]	130 ± 5
TNF-α (ng/g fresh tissue)	0.1 ± 0.0	1.2 ± 0.1[a]

Colonic parameters were quantified in the control group ($n = 10$), which received saline instillation at day 0. TNBS group ($n = 12$) received trinitrobenzene sulphonic acid intracolonically at day 0 in a vehicle of 40% ethanol; control group received 40% ethanol intracolonic injection at day 0. Data are expressed as mean ± S.E.M.

[a] $P < 0.05$ by comparison with control group.

[b] Not detected.

Table 3
Ex vivo inhibition of TNF-α production from colon inflamed mucosa by trinitrobenzene sulphonic acid/ethanol intracolonic instillation

Compound	Concentration (μM)	TNF-α inhibition (%)
Dexamethasone	0.01	26.6 ± 8.2
	0.1	65.2 ± 3.7[a]
	1	84.5 ± 6.8[a]
	10	89.9 ± 5.5[a]
Sulfasalazine	50	14.5 ± 4.8
	100	47.4 ± 4.5[a]
	200	55.0 ± 8.6[a]
JM 34 maleate	50	24.0 ± 3.9
	100	49.0 ± 9.5[a]
	200	50.2 ± 4.6[a]
	300	85.0 ± 6.6[a]
XC 21	1	19.2 ± 4.9
	10	32.0 ± 6.5[a]
	50	40.8 ± 9.9[a]

At day 5, inflamed colonic pieces were cultured for 5 h in RPMI 1640, foetal calf serum 10% without inhibitor (trinitrobenzene sulphonic acid group) or in the presence of different agents at various final concentrations: dexamethasone (0.01, 0.1, 1 and 10 μM), sulphasalazine (50, 100 and 200 μM), JM 34 maleate (50, 100, 200 and 300 μM), and XC 21 (1, 10 and 50 μM). Data are expressed as a percentage of the inhibition of TNF-α production (mean ± S.E.M.).

[a] $P < 0.05$ by comparison with TNBS group.

Trinitrobenzene sulphonic acid instillation induced a marked increase of all studied parameters. Lesions in the distal colon were quantified by a macroscopic damage score (mean: 7.7 ± 1.2 and min–max: 6–9). These macroscopical lesions were associated with substantial myeloperoxidase activity (130 ± 5 mIU/mg), and TNF-α production was 1.2 ± 0.1 ng/g of fresh tissue.

3.2. Ex vivo inhibition of TNF-α mucosal production

Table 3 summarises the percentages of inhibition of the four anti-inflammatory agents on TNF-α mucosal production ex vivo 5 days after trinitrobenzene sulphonic acid/ethanol instillation. Ex vivo TNF-α production of inflamed colon was 1.2 ± 0.1 ng/g of fresh tissue. Dexamethasone was the most potent agent, producing more than 80% TNF-α inhibition, even at low concentrations (1 and 10 μM). JM 34 maleate, sulphasalazine, and XC 21 produced 60% inhibition or less at concentrations below 200 μM.

3.3. Effects of chronic treatment of anti-inflammatory drugs on trinitrobenzene sulphonic acid-induced colitis

Diarrhoea was severe after chronic treatments with dexamethasone (1 mg/kg/day) and JM 34 maleate (100 mg/kg/day), but disappeared at day 3. The behaviour of animals treated with these two agents was similar to that of the untreated group during the first 3 days. However,

sulphasalazine and XC 21 treatments caused only minor diarrhoeic symptoms, which disappeared 1 or 2 days after colitis induction. After these last two treatments, the behaviour of rats was not modified.

Fig. 1 indicates the effects of chronic treatment (8 days) of the tested compounds on trinitrobenzene sulphonic acid/ethanol-induced colitis as rated by the macroscopic damage score (mean: 7.2 ± 0.3). No damage was quantified in the control group. The damage score after JM 34 maleate treatment was moderately lower (6.3 ± 0.6), whereas the other chronic treatments (dexamethasone, sulphasalazine, and XC 21) induced a significant decrease (between 3.9 and 4.0) compared to the trinitrobenzene sulphonic acid group ($P < 0.05$). No significant differences were observed between these treatments.

The colonic myeloperoxidase activity of trinitrobenzene sulphonic acid and of animals treated with trinitrobenzene sulphonic acid is indicated in Fig. 2. All four agents reduced myeloperoxidase activity significantly compared to the untreated group. In the latter group ($n = 12$), which received vehicle (oral gavage daily of 0.2 ml/100 g of methylcellulose 1%), mean myeloperoxidase activity was 115.3 ± 6.3 mIU/mg of colonic mucosal protein. In treatment groups, this parameter was markedly decreased after dexamethasone ($n = 9$) and JM 34 maleate ($n = 9$) to 82.0 ± 9.2 and 71.3 ± 10.2 mIU/mg, respectively. The lowest trinitrobenzene sulphonic acid activities were noted after chronic sulphasalazine ($n = 9$) and XC 21 treatment ($n = 9$): 37.2 ± 11.4 and 22.4 ± 8.2 mIU/mg of colonic mucosal protein, respectively. XC 21 decreased myeloperoxidase activity significantly compared to dexamethasone and JM 34 maleate treatments.

TNF-α production of animals treated or not with trinitrobenzene sulphonic acid was quantified immediately after necropsy. Fig. 3 shows that intracolonic administration of trinitrobenzene sulphonic acid resulted in marked TNF-α production 8 days after colitis induction (0.90 ± 0.01 ng/g of fresh tissue). All treated rats exhibited a significant decrease ($P < 0.05$) of TNF-α mucosal production. The dexamethasone-treated trinitrobenzene sulphonic acid group showed a mean level of 0.35 ± 0.09 ng/g of TNF-α after chronic treatment (1 mg/kg/day). At the concentrations used, the groups treated with sulphasalazine, JM 34, and XC 21 showed a marked decrease in colonic mucosal

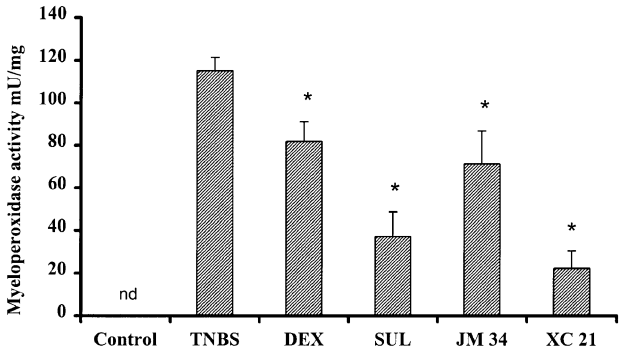

Fig. 2. Effect of chronic treatment by anti-inflammatory drugs on myeloperoxidase activity of colon 8 days after trinitrobenzene sulphonic acid/ethanol instillation. Colonic mucosal myeloperoxidase activity was quantified in the absence of treatment, but with daily administration of the vehicle methylcellulose 1% (trinitrobenzene sulphonic acid group, $n = 12$), or in the presence of dexamethasone 1 mg/kg/day (DEX group, $n = 9$), sulphasalazine 100 mg/kg/day (SUL group, $n = 9$), JM 34 maleate 100 mg/kg/day (JM 34 group, $n = 9$), and XC 21 50 mg/kg/day (XC 21 group, $n = 9$). Data are expressed as the mean ± SEM. The myeloperoxidase activity of colonic mucosa was quantified as described in Materials and methods and expressed as mU/mg of protein.

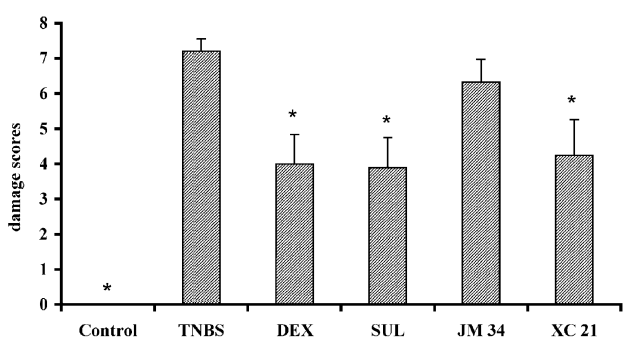

Fig. 1. Effects of chronic administration of various drugs on the colonic damage score. Colonic macroscopic damage resulting from trinitrobenzene sulphonic acid/ethanol instilled into rat colon was scored as indicated in Table 1. Scores were quantified in the absence of treatment, but with daily administration of the vehicle methylcellulose 1% (trinitrobenzene sulphonic acid group, $n = 12$), or in the presence of dexamethasone 1 mg/kg/day (DEX group, $n = 9$), sulphasalazine 100 mg/kg/day (SUL group, $n = 9$), JM 34 maleate 100 mg/kg/day (JM 34 group, $n = 9$), and XC 21 50 mg/kg/day (XC 21 group, $n = 9$). Data are expressed as the mean ± SEM ($^*P < 0.05$).

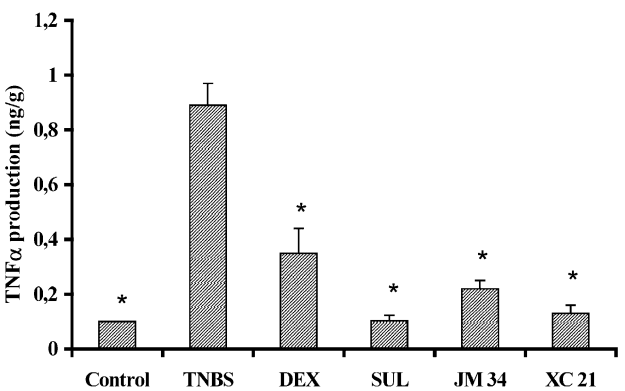

Fig. 3. Effect of chronic treatment by anti-inflammatory drugs on TNF-α colonic mucosa production 8 days after trinitrobenzene sulphonic acid/ethanol instillation. Colonic mucosal TNF-α production was quantified in the absence of treatment, but with daily administration of the vehicle methylcellulose 1% (TNBS group, $n = 12$), or in the presence of dexamethasone 1 mg/kg/day (DEX group, $n = 9$), sulphasalazine 100 mg/kg/day (SUL group, $n = 9$), JM 34 maleate 100 mg/kg/day (JM 34 group, $n = 9$), and XC 21 50 mg/kg/day (XC 21 group, $n = 9$). Data are expressed as the mean ± SEM. TNF-α production of colonic mucosa was quantified as described in Materials and methods.

production of TNF-α: 0.10 ± 0.02, 0.22 ± 0.03, and 0.13 ± 0.03 ng/g fresh tissue, respectively. As in the case of myeloperoxidase activity, sulphasalazine and XC 21 appeared to be the most efficient treatments, markedly inhibiting trinitrobenzene sulphonic acid induced TNF-α production as compared to dexamethasone and JM 34 maleate ($P < 0.05$).

4. Discussion

Trinitrobenzene sulphonic acid/ethanol-induced colitis is a useful animal inflammation model in the rat and of particular interest in relation to Crohn's disease (transmural infiltration, discontinuous damage). According to Allgayer et al. (1989), transmural necrosis (a sign of acute inflammation) disappears about 7 days after trinitrobenzene sulphonic acid/ethanol instillation. Thus, day 8 may be considered as representing a chronic state of inflammation (Rachmilewitz et al., 1989).

Colitis induction by trinitrobenzene sulphonic acid is associated with notable body weight loss during the first 5 days. In some experiments using high trinitrobenzene sulphonic acid concentrations, body weight loss persisted up to 7 days (Boughton-Smith et al., 1988). Weight loss in ulcerative colitis is mainly due to abdominal pain and anorexia.

In the present study, the development of trinitrobenzene sulphonic acid-induced colitis was studied in relation to the macroscopic features of inflamed tissue (macroscopic damage score) and biochemical parameters (myeloperoxidase activity of colonic mucosa and TNF-α production). Macroscopic scores were used to quantify the severity of inflammation. The extent of colonic damage is dependent on the initial concentration instilled into the rat colon, which could account for discrepancies between different studies (Yue et al., 1996). Myeloperoxidase activity provides a quantitative index of disease severity and a method of assessing drug efficacy in animal models of intestinal inflammation (Krawitz et al., 1984). The increase of myeloperoxidase activity occurs only in the region directly exposed to trinitrobenzene sulphonic acid instillation and decreases within 7 days.

In our experiments, myeloperoxidase activity and TNF-α production in inflamed colon were determined on a whole piece of tissue. Different experimental procedures have been described to quantify these parameters. However, according to recent studies, TNF-α in the acute stage of trinitrobenzene sulphonic acid inflammation is apparently produced by muscle cells, which confirms our data based on incubation of total mucosa and not simply on a scraping (Lamrani et al., 1999; Yue et al., 1996). As this cytokine is very labile, serum TNF-α concentrations are negligible 1 week after colitis induction (Barbier et al., 1998; Applyard and Wallace, 1995).

Sulphasalazine administered daily appears to be the most potent agent, according to our results and those of Sykes et al. (1999). Classical non-steroidal anti-inflammatory drugs such as indomethacin and naproxen, in dose-dependent conditions, increase the severity of colonic damage and the incidence of mortality in rats injected with trinitrobenzene sulphonic acid/ethanol. This effect is probably correlated with the increase of TNF-α production induced by these drugs (Wallace et al., 1992). Like other aminosalicylates, sulphasalazine appears to be an inhibitor of nuclear factor κB (NFκB) activation (Wahl et al., 1998). This type of inhibition appears to be the main, although not exclusive, effect of its anti-inflammatory mechanism, as sulphasalazine is also a potent inhibitor of lipo-oxygenase and cyclo-oxygenase pathways and blocks platelet aggregating factor activity and potent oxygen radical scavengers (Nikolaus et al., 2000).

Dexamethasone, a reference drug used as an inhibitor of NFκB activation (Auphan et al., 1995), was unable to improve digestive disturbance during the first stage of inflammation (diarrhoea) as described by Kojouharoff et al. (1997). However, after these first 3 days, dexamethasone treatment improves clinical scores. The efficiency of dexamethasone in inhibiting TNF-α production in vitro is well known, and our ex vivo results demonstrate once again the potency of this agent as compared to the salicylate derivative. When tested intraperitoneally on trinitrobenzene sulphonic acid-induced colitis, chronic treatment with dexamethasone 1 mg/kg, as well as 5-aminosalicylic acid, prevented an increase in the colonic damage score and in myeloperoxidase activity (Applyard and Wallace, 1995). Our results confirm these findings and also show a decrease of TNF-α colonic mucosal production. Similar data have been reported for Crohn's disease (Geboes et al., 1999).

In previous studies, our group showed the efficiency of XC 21 (the phthalimide derivative) in inhibiting TNF-α production in vitro (around 80% of TNF-α with a concentration of only 10 μM on lipopolysaccharide-stimulated macrophages) (Collin et al., 1998). Moreover, XC 21, when evaluated on the well-known carrageenan-induced oedema model, decreased inflammation by 80% after oral gavage of 50 mg/kg. According to ex vivo results in the present study, XC 21 was unable to inhibit TNF-α production on a "well-established" inflamed tissue. However, chronic XC 21 therapy appeared to stimulate colonic repair, as evidenced by clinical recovery and decreased inflammation with respect to TNF-α and myeloperoxidase activities. XC 21 improved clinical signs from the first days of colitis induction. The efficiency of thalidomide compounds was suggested as early as 1979, and thalidomide was used to treat ulcerative colitis in humans (Waters et al., 1979). The improvement profile of this drug is close to that of the reference agent sulphasalazine. In the present study, the phthalimide derivative XC 21 improved all clinical and biochemical parameters studied when a dose

half that of the reference agent sulphasalazine was used. As expected, good correlation was obtained between inhibition in vitro of TNF-α synthesis and efficiency in a well-established in vivo TNF-α-dependent model. However, the mechanism of action and the potential adverse effects of XC 21 still need to be evaluated.

In ex vivo studies of JM 34 maleate (a heterocarboxamide derivative), the concentrations used were based on previous tests in vitro (Robert et al., 1995). The dose range of chronic treatments was selected according to the efficiency of the compounds in carrageenan-induced oedema, the classical inflammation model in rats. JM 34 maleate 200 μM induced 80% inhibition of TNF-α, but our ex vivo results suggest that it is unable to inhibit TNF-α production totally on a "well-established" inflamed tissue. However, daily administration had attenuated the biochemical factors (myeloperoxidase activity and TNF-α production) when colonic damage was assessed at 8 days. As in the case of dexamethasone, chronic treatment with JM 34 maleate was unable to improve the macroscopic damage score or clinical manifestations on trinitrobenzene sulphonic acid-induced colitis in rats. Eight days after induction, colonic damage was close to that described for trinitrobenzene sulphonic acid in control animals. This delay in healing may have been due to specific cyclo-oxygenase 2 inhibition, as recently reported (Lesh et al., 1999). The inhibition capacity of this heterocarboxamide derivative on cyclo-oxygenase 2 needs to be evaluated, especially with regard to recent findings (Willoughby et al., 2000). Thus, JM 34 maleate gave unexpected results, failing to improve trinitrobenzene sulphonic acid-induced colitis in rats. Reduced TNF-α mucosal production was not associated with a decrease of myeloperoxidase and the macroscopic damage score. Studies are in progress in our laboratory to determine the exact pharmacological mechanisms relating to JM 34 maleate.

The similar results obtained with dexamethasone and JM 34 maleate suggest that the latter compound, which partially inhibits MAP-kinase (Vernhet et al., 1997), could interfere with other transductional mechanisms (Brown et al., 1999; Yin et al., 1998).

In summary, trinitrobenzene sulphonic acid-induced colitis, a well-known animal inflammation model, was studied to test the anti-inflammatory potency of active TNF-α synthesis inhibitors in vitro. This model was validated in accordance with clinical and biochemical parameters. Our results confirm the efficiency of oral chronic treatment with the phthalimide derivative XC 21, which provided effects similar to those of the reference agent sulphasalazine with respect to clinical and biochemical parameters. JM 34 maleate (a heterocarboxamide derivative) failed to improve colonic damage, although decreased myeloperoxidase activity and TNF-α production were observed. On the basis of these results, further studies are in progress to determine the specific mechanisms of action of these two compounds.

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