Treatment with tumour necrosis factor inhibitor oxpentifylline does not improve corticosteroid dependent chronic active Crohn's disease

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Abstract

Background—In Crohn's disease, inflammation is presumably sustained by an increased production of proinflammatory cytokines, in particular tumour necrosis factor α (TNF α) and interleukin 1 β (IL1 β). TNF α can induce a host of cellular effector events resulting in perpetuation of the inflammatory process. In vivo studies with anti-TNF α antibody treatment have led to impressive clinical results.

Aims—To investigate whether treatment with the TNF α inhibitor oxpentifylline results in clinical improvement in corticosteroid dependent chronic active Crohn's disease.

Methods—Sixteen Crohn's disease patients received oxpentifylline 400 mg four times a day in a four week open label study.

Results—Blockade of TNF α production in 16 patients with corticosteroid dependent Crohn's disease did not improve the clinical disease activity (CDAI mean (SEM) 188.75 (5.65) versus 185.13 (10.87) or the endoscopic degree of inflammation (CDEIS 14.9 (2.87) versus 14.8 (2.27) or laboratory parameters.

Conclusions—In this study, use of the TNF α inhibitor oxpentifylline does not improve inflammation in Crohn's disease. This finding suggests that there may be more key mediators than only TNF α in the inflammatory process in Crohn's disease.

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Keywords: Crohn's disease, intestinal immunity, tumour necrosis factor α , inflammation, oxpentifylline.

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Recent studies have convincingly demonstrated that an increased release of proinflammatory cytokines by intestinal lamina propria mononuclear cells is involved in the perpetuation of intestinal inflammation in inflammatory bowel disease (IBD).¹⁻⁸ Intestinal as well as peripheral mononuclear cells are highly activated during acute inflammatory bowel disease and hence capable of releasing increased amounts of several proinflammatory mediators including tumour necrosis factor α (TNF α) and interleukin 1 β (IL-1 β).¹⁻⁸ In vitro findings show that TNF α is capable of inducing a host of proinflammatory effector events, which are thought to be implicated in the pathophysiology of IBD. TNF α has been shown to be involved in neutrophil accumulation,⁹ granuloma formation,^{10 11} upregulation of adhesion mole-

cules on endothelial cells,¹² procoagulant effects,¹³ induction of increased intestinal permeability,¹⁴ and also has direct cytopathic effects.¹⁵ TNF α concentrations in serum¹⁶ as well as stool¹⁷ and intestinal mucosa^{1 2 5} were found to be raised in patients with active Crohn's disease, in comparison with both normal controls and inactive disease.^{1 2 18 19} However, increased transcription and translation of TNF α is not specific for IBD.^{20 21} In other studies, Isaacs *et al* could not detect TNF α messenger RNA in a considerable number of biopsy specimens from patients with IBD,⁷ and Hyams *et al*²² did not report increased TNF α values in patients with IBD.

The hypothesis that TNF α may be of particular importance in the induction and perpetuation of intestinal inflammation in IBD and consequently suppression of TNF α should improve inflammation in patients with Crohn's disease was strongly supported by a series of recent therapeutic studies.^{23 24} Experimental treatment of corticosteroid dependent patients with active Crohn's disease by a one time application of a humanised monoclonal antibody directed against TNF α (cA2 IgG1) has generated impressive clinical results. Within four weeks, clinical and endoscopic inflammation completely resolved in eight of 10 patients.²⁴ Other pilot studies using a different monoclonal antibody (CDP571) directed against TNF α could not generate such impressive results.²⁵ The CDP571 antibody is from the IgG4 subclass and is therefore theoretically less capable to activate complement in comparison with an IgG1 antibody.

If reduction of TNF α is the exclusive mechanism of action of the TNF α cA2 antibody treatment, other drugs that also reduce TNF α should have similar effects. Oxpentifylline (pentoxifylline, PTX), a xanthinoxidase inhibitor, is a strong suppressor of TNF α release by a host of different cell populations in vitro as well as in vivo.²⁶⁻³³ In dose between 1200 mg/d and 2000 mg/d, PTX was a strong inhibitor of TNF α release in vivo and effective in improving TNF α related inflammatory disease.^{27-29 33} The presence of corticosteroids potentiates the TNF α inhibitory effect of PTX.³⁴ However, as other phosphodiesterase inhibitors PTX has additional contrainflammatory effects,^{30 35} some of which cannot be restored by addition of TNF α in vitro.³⁰

We treated 16 patients with stable, corticosteroid dependent Crohn's disease with PTX at a dose of 400 mg four times daily in an open label study in which we found no improvement

of intestinal inflammation or clinical symptoms. The discordance of our findings and those by Dulleman *et al.*^{23, 24} raises important questions regarding the mechanism of cA2 anti TNF α antibody treatment.

Methods

PATIENTS

Chronic active corticosteroid dependent Crohn's disease was defined by a Crohn's disease activity index (CDAI) between 150–250 and at least 10 mg prednisone daily for a minimum of three months. Patients had to have at least one active episode requiring acute phase treatment³⁶ in the preceding six months. Diagnosis of Crohn's colitis or ileocolitis involving at least 30 cm of the large bowel had to be previously established by radiological, endoscopic, or clinical criteria,³⁷ or all three. Only mesalazine (up to a dose of 1 g thrice daily) and corticosteroids were permitted as anti-inflammatory treatment and had to be kept stable two weeks before the study. Use of loperamide or codeine to control diarrhoea was permitted and recorded for calculation of the CDAI. No pain medications, other than tramadol, nutritional therapy (parenteral, formula diets) or immunosuppressives (within the preceding six weeks), were permitted.

Exclusion criteria were bacterial or parasitic pathogens in the patients' stools, a positive *Clostridium difficile* toxin test, clinical signs of septicaemia, intestinal perforation, megacolon, history of resections other than an ileocaecal resection, signs of stenosis, active fungal or viral infection or when it was felt that patients could not be maintained stable with their present therapeutic regimen for the time of study. Patients were also excluded if they had raised transaminase activities (>3 times normal), hyperbilirubinaemia (>2 times normal), signs of renal dysfunction (serum creatinine >33% increased) or a serum cholesterol concentration of less than 100 mg/dl. Informed consent was obtained from all patients. The study was given approval by the local ethics review committee.

Of 152 patients with Crohn's disease seen in the outpatient clinics, 47 patients with chronic active Crohn's disease were screened for inclusion in the study. Of these, 31 patients were excluded for several reasons: a history of

bowel surgery other than ileocaecal resection (9), because it was not expected that their corticosteroid treatment would remain stable throughout the study (7), clinical signs of stenosis (6), positive *Clostridium difficile* toxin testing (1), increased transaminases and bilirubin (1), and signs of renal dysfunction (2). Five patients refused to enter the study. The remaining 16 patients (Table) received oxpentifylline.

BASELINE STUDIES AND FOLLOW UP

A clinical visit was scheduled two weeks before the tentative start of oxpentifylline treatment and colonoscopy performed within one week before enrolment.

The ability to perform social functions was estimated with a questionnaire according to Robinson *et al.*³⁸ The social function questionnaire consisted of the following seven questions: How much do you feel affected by your disease: (1) in job related activities, (2) in everyday activities in your home, (3) in private activities outside your home, (4) in your general social contacts, (5) in your hobbies/spare time, (6) in sexual activities, and (7) during sleep. Patients were instructed to mark their answer on a horizontal line of 7 cm length with the far left side indicating that the social function asked for was not affected and the far right side indicated that it was maximally affected. Measuring the distance from the far left side to the marker the patient set, values could be obtained which reached from 0 (fully capable of performing the social function) to 7 (intense suffering from a total disability to perform the social function).

All patients received oxpentifylline (pentoxifylline (PTX), Trental, Hoechst AG, Frankfurt, Germany) at a dose of 400 mg four times daily orally. This dose range is established as inhibitory for TNF release by trials in AIDS,²⁸ bone marrow transplantation²⁷ as well as in healthy volunteers.²⁹ Patients were seen two and four weeks after start of PTX treatment and at each of these time points, laboratory tests were performed (including CRP and C reactive protein erythrocyte sedimentation rate), a physical examination carried out, and the CDAI calculated. At the four week time point the questionnaire, pertaining to the patient's social functions,³⁸ was repeated. After four weeks PTX

Clinical data of patients

Patient	Age (y)	Sex	Duration of Crohn's disease (y)	CDAI	CDEIS	Ileocaecal resection	Corticosteroid treatment (months)	Prednisone dose (mg)	Extraintestinal manifestations	Mesalazine	Ileum involved
1	23	M	4	167	12.4	No	7	10	—	Yes	Yes
2	19	F	7	188	15.8	No	9	15	arthralgia	Yes	Yes
3	24	F	5	202	14.8	Yes	17	15	erythema nodosum	Yes	Yes
4	20	M	2	187	13.2	No	12	10	—	Yes	No
5	27	M	8	190	11.7	No	90	10	arthralgia	Yes	Yes
6	31	F	5	212	13.4	No	7	10	arthritis	Yes	Yes
7	56	F	24	232	17.8	Yes	144	20	arthralgia	No	Yes
8	47	F	17	170	16.2	No	60	15	—	Yes	Yes
9	42	M	2.5	205	20.2	Yes	10	20	—	Yes	Yes
10	41	F	4.5	212	15	No	14	25	arthralgia	Yes	No
11	37	F	11	207	13.4	No	66	20	—	Yes	Yes
12	25	M	9	165	18.4	No	5	15	—	Yes	Yes
13	18	F	2	152	19	No	6	10	—	Yes	Yes
14	22	F	1.5	178	9.6	No	6	10	—	Yes	No
15	34	M	7	202	11.6	No	48	15	arthritis	Yes	Yes
16	29	F	10	151	15.4	No	30	20	—	Yes	Yes

medication was stopped and a repeat colonoscopy was performed (trial end ± 3 days).

Patients were informed to increase or decrease their corticosteroid dose in a range of ± 10 mg on demand. Corticosteroid use was protocolled in patients diaries. All patients who worsened during the trial (by an increase in CDAI of >50 points or by physicians global assessments), were treated appropriately by increasing the daily dose of prednisone. In this case PTX was stopped. Patients who stopped their medication because of adverse reaction (none) or treatment failure (four), were followed up in the same way as those who continued to receive PTX. The primary outcome measure was the induction of clinical remission, as defined by decrease of the CDAI below 150, but at least by 50 points. Secondary outcome measures were changes in CDAI, Crohn's disease endoscopic index (CDEIS),³⁹ the ability to reduce prednisolone treatment, and improvement of social functions.

IN VITRO CYTOKINE STUDIES

Fetal calf sera and pokeweed mitogen were purchased from Gibco (Grand Island, NY). $\text{TNF}\alpha$, $\text{IL1}\beta$, and IL6 ELISA kits were obtained from R&D/DPC Biemann (Bad Nauheim, Germany). All other chemicals were obtained from Sigma if not specified differently. Peripheral blood mononuclear cells (PBMNC) or peripheral blood monocytes were isolated as previously described,⁴⁰ cultured in the presence of pokeweed mitogen (1% vol/vol, 24 hours), and supernatant cytokine values determined in duplicate by ELISA.

STATISTICS

Results are expressed as mean (SEM). Statistical significance of differences was tested by non-parametric Spearman correlation⁴¹ or the Mann-Whitney U test.

Results

DISEASE ACTIVITY AND INFLAMMATION PARAMETERS

CDAI scores did not change significantly during the treatment period (188.75 (5.65) before versus 185.13 (10.87) after treatment; Figure). Four patients stopped PTX treatment because of increased disease activity. None of the patients could discontinue prednisone during PTX treatment, five patients reduced prednisone by 5 mg daily, and five patients increased prednisone use. Overall, prednisone use on a cumulative basis was not changed significantly by PTX treatment (15 (4.68) before (n=16), versus 12.5 (2.5) after treatment (n=12)).

Crohn's disease endoscopic index before and after treatment with PTX did not change significantly (14.9 (2.87) before treatment (n=16) versus 14.8 (2.27) after treatment (n=12), Figure). All four patients who had to stop PTX treatment because of increased clinical activity, did have higher than average CDEIS scores at study begin (16.6 mean).

CDEIS and CDAI under treatment with oxpentifylline. CDEIS scores before and after treatment with oxpentifylline appeared to be unchanged. (A) Average CDEIS scores were 14.9 (2.87) before (n=16) and 14.8 (2.27) after treatment (n=12). In four patients oxpentifylline had to be stopped after two weeks because of an increased disease activity. (B) CDAI scores before treatment with oxpentifylline ranged between 150–250. During the trial no significant improvement was observed.

Serum concentrations of C reactive protein (27.1 (14.7) before treatment versus 35.2 (17.5) after treatment) and erythrocyte sedimentation rate (22.8 (4.6) before treatment versus 28.6 (5.4) after treatment) did not change significantly.

None of the patients receiving oxpentifylline treatment experienced severe side effects.

EFFECTS OF PTX ON $\text{TNF}\alpha$ PRODUCTION

In six patients $\text{TNF}\alpha$ secretion by peripheral monocytes was studied before and at the end of PTX treatment (35.8 (13.9) pg/ml before treatment versus 13.2 (19) after treatment, non-significant). All patients had detectable $\text{TNF}\alpha$ production before PTX, in five patients it decreased below the sensitivity range, although in two patients values were already at the lower detection limit before PTX treatment. In one patient $\text{TNF}\alpha$ release was not inhibited by PTX.

Five healthy volunteers received oxpentifylline 400 mg four times daily for a period of two days. Release of $\text{TNF}\alpha$ by 10^6 PBMNC/ml stimulated with lipopolysaccharide (1 $\mu\text{g}/\text{ml}$ for 24 hours) was significantly suppressed by in vivo PTX in comparison with baseline values (1068 (198) before, 251 (122) pg/ml after two days oxpentifylline; $p=0.008$). In contrast, secretion of $\text{IL1}\beta$ (324 (66) versus 350 (62) pg/ml) and IL6 (2124 (311) versus 2252 (225) pg/ml) did not change significantly.

SOCIAL FUNCTION SCORES

Social function scores showed that co-treatment with oxpentifylline did not significantly improve the patient's social functions except for sleep disturbance (week 0: 3.6 (0.35) (n=16), score week 4: 2.4 (0.29) (n=12), $p=0.017$). Upon detailed interviewing most patients indicated retrospectively that improvement of sleep was due to reduced muscle aches and reduced night sweat.

Discussion

Chronic inflammatory activity in Crohn's disease may be sustained by the local release of proinflammatory cytokines from intestinal macrophages and T cells including secretion of TNF α , IL1 β , and IL6.^{3 7 13 14 20-21} As TNF α is a potent proinflammatory mediator, which can be released by mononuclear phagocytes, activated T cell subpopulations as well as invading granulocytes⁴² the specific blockade of TNF α has been considered a promising approach for treatment of intestinal inflammation.^{23 24} Strong support for this hypothesis has been given by experimental treatment of chronic active corticosteroid dependent Crohn's disease with a one time application of a monoclonal antibody (cA2) directed against TNF α , which induced complete endoscopic and clinical remission in eight of 10 patients within four weeks.^{23 24} These findings may indicate a pivotal role of TNF α in the pathophysiology of intestinal inflammation in Crohn's disease.

To test this hypothesis we specifically blocked TNF α production by another agent, oxpentifylline, which is an established inhibitor of TNF α release in vitro as well as in vivo.²⁶⁻³⁴ Oxpentifylline has therefore been used in dose from 1200 to 2000 mg/d in clinical trials to suppress presumable in vivo effects of TNF α .^{27-29 33} However, in common with other phosphodiesterase inhibitors, PTX may have also additional contrainflammatory effects including a reduction of T lymphocyte cytokine secretion (that is, IFN γ) in vitro.^{30 35}

In contrast with anti-TNF α cA2 antibody treatment no effect by PTX could be seen on clinical, laboratory or endoscopic activity. With the exception of sleep disturbance by the disease, none of the social functions assessed by a questionnaire improved. However, upon a detailed interview most patients indicated retrospectively that improvement of sleep was due to reduced muscle aches and reduced night sweat. These are both symptoms that may be attributed to raised TNF α levels.⁴³

The negative results of PTX treatment suggest that the mechanism of anti-TNF α antibody treatment (using the monoclonal antibody cA2^{23 24} supplied by Centocor, PA, USA) in Crohn's disease may not only relate to a specific blockade of TNF α secretion. Other mechanisms including complement mediated lysis of cells expressing membrane bound TNF α ^{44 45} may possibly contribute to its efficacy. Thus, the cA2 antibody could also inhibit mediators apart from TNF α ,⁴⁶ which may sustain chronic intestinal inflammation. Taken together, our findings and the findings

of Derkx *et al*^{23 24} may indicate that there are more key mediators than only TNF α in the inflammatory process in Crohn's disease.

It is not known how much suppression of TNF α is actually induced in the mucosal compartment by either cA2 anti-TNF antibody treatment or by PTX. Moreover, the possibility exists that a substantial proportion of mucosal TNF α originates from T cells, and inhibition of TNF production by mucosal T cell populations by PTX has not been investigated yet. Finally, although selection criteria were similar in both trials, Derkx *et al*^{23 24} investigated in their uncontrolled pilot study a more active patient population (as indicated by the higher average CDAI) than we did. Therefore, patients' mucosal TNF α levels may have been different between both trials.

Further studies are necessary to clarify the relevance of mucosal TNF α production in the pathophysiology of inflammation in Crohn's disease. In this pilot trial, oxpentifylline at a dose of 400 mg four times daily given over a period of four weeks was not effective in treating patients with corticosteroid dependent chronic active Crohn's disease. Further controlled studies are warranted to examine the therapeutic potential of oxpentifylline and other TNF α inhibitors in Crohn's disease.

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