An open study of pentoxifylline and thalidomide as adjuvant therapy in the treatment of rheumatoid arthritis

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Abstract

Objective—Dysregulation of tumour necrosis factor α (TNF α) production is thought to be important in rheumatoid arthritis. Since pentoxifylline and thalidomide inhibit endotoxin induced TNF production in vitro, these drugs were tested in an open study in rheumatoid arthritis patients to assess toxicity, the effect on TNF production, and the antiarthritic effects.

Methods—12 patients with active rheumatoid arthritis were treated with 1200 mg pentoxifylline and 100 mg thalidomide a day during 12 weeks. In addition, TNF production was assessed by ex vivo whole blood cultures stimulated with endotoxin.

Results—Adverse events such as xerostomia, drowsiness, and constipation occurred in almost all patients, which led to discontinuation in three. The drugs halved the TNF production capacity during treatment (ANOVA, $P < 0.03$) whereas production capacity of interleukin (IL) 6, IL-10, and IL-12 was not affected. Of the nine patients who completed the study, five fulfilled the ACR -20% response criteria after 12 weeks of treatment.

Conclusions—Although pentoxifylline/thalidomide reduced the production capacity of TNF, the benefit/side effects ratio was poor due to multiple adverse effects, while clinical observation suggests limited efficacy.

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reduced clinical indices of inflammation in open studies in various disease states such as bone marrow transplantation,⁴ AIDS,⁵ and cerebral malaria.⁶ In an open study in rheumatoid arthritis patients, Maksymowych *et al*⁷ showed that pentoxifylline has both a moderately beneficial clinical response and an inhibitory effect on TNF production capacity.

Thalidomide is a drug that selectively inhibits TNF production by monocytes, presumably by enhancing degradation of messenger RNA (mRNA).^{8,9} In various groups of patients, for example those suffering from discoid lupus erythematosus, prurigo nodularis, recurrent aphthosis, Jessner's lymphocytic infiltration, pulmonary tuberculosis,¹⁰ chronic graft versus host disease,¹¹ and rheumatoid arthritis,¹² open studies have been performed in which thalidomide reduced clinical indices of inflammation. In severe aphthosis the beneficial effects of thalidomide were confirmed in a placebo controlled study.¹³

The mechanisms underlying the inhibition of TNF α production by pentoxifylline and thalidomide are theoretically synergistic. We therefore carried out an open trial in rheumatoid arthritis patients to determine whether the combination of pentoxifylline and thalidomide was toxic, effective with regard to reduction of the endotoxin induced TNF production ex vivo, and had an antiarthritic effect.

Methods

STUDY DESIGN

Patients received pentoxifylline 400 mg three times daily and 100 mg thalidomide once a day in an open study over 12 weeks. During the study, existing antirheumatic drug treatment was continued.

PATIENT POPULATION

Twelve patients with rheumatoid arthritis according to the 1987 criteria of the American Rheumatism Association were recruited from the outpatient clinic of the Academic Hospital Leiden, The Netherlands. Patients were included if they met the inclusion criteria for active disease, defined by the presence of six or more swollen joints (out of 28) and at least two of the following criteria: (1) the presence of nine or more joints that are painful on motion or tender on pressure; (2) morning stiffness lasting at least 45 minutes; (3) an erythrocyte sedimentation rate (ESR) of at least 28 mm during the first hour. Exclusion criteria were a history of peripheral sensory neuropathy,

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The identification of tumour necrosis factor α (TNF α) as one of the key mediators of inflammation in rheumatoid arthritis has led to randomised trials using anti-TNF α monoclonal antibody (Mab) which were reported to have beneficial results.^{1,2} These results support the hypothesis that reducing TNF concentrations is an attractive goal in the treatment of rheumatoid arthritis.

TNF is an inducible cytokine that is not produced in resting cells. Stimulation of monocytes by endotoxin is used as a model system to select drugs that inhibit TNF production. Pentoxifylline, a methylxanthine derivative and phosphodiesterase inhibitor, reduces TNF production in cultured monocytes by inhibition of transcription of the TNF α gene.³ In patients, pentoxifylline

Table 1 Changes in production of cytokines in whole blood cultures

	Week No					ANOVA test P
	0	4	8	12	16	
Stimulus 100 ng endotoxin TNF (measured by ELISA)	9.1 (7)	4 (5)	4.1 (4.6)	11 (7)	10 (5)	0.005
Stimulus 1000 ng endotoxin TNF (measured by ELISA)	9.8 (8)	5.3 (6.4)	8.2 (13.3)	16.5 (9.9)	14.2 (9.8)	0.011
TNF (measured by bioassay)	21 (3)	14.8 (5.4)	16.4 (16.7)	26.1 (3.8)	22 (3.3)	0.00
IL6 (measured by ELISA)	39 (15)	30 (18)	28 (23)	33 (17)	32 (17)	0.8
IL10 (measured by ELISA)	7.5 (4.3)	8.1 (5.4)	9.2 (6.6)	12.5 (7.8)	8.7 (7.6)	0.37
IL12 (p40 subunit measured by ELISA)	12.5 (4.7)	11.6 (10)	10 (8)	10.3 (8.8)	12.8 (6.4)	0.7

active gastroduodenal ulcer, severe constipation, vertigo of vestibular disease, females with childbearing potential, or a change in the antirheumatic treatment during the preceding three months.

EVALUATION

Patients

Clinical and laboratory assessments were performed at the start of the study, at week 4 and 8 during the study, at week 12 one day after the study, and 4 weeks after discontinuation of the treatment. The following clinical indices were assessed:

- (1) The number of swollen joints (out of 28)
- (2) The number of tender joints
- (3) The number of joints with restricted motion
- (4) The grip strength using a dynamometer
- (5) The physician's and the patient's global assessment of disease activity on a scale of 0-10.

Patients were always evaluated between 9 and 11 am and phlebotomy was performed at that time. Patients were also evaluated as responders or non-responders according to the modified American College of Rheumatology (ACR) core set of improvements.

Cytokine production

TNF α , interleukin (IL)10, IL-12, and IL-6 production capacity was evaluated using a whole blood stimulation assay as described. The concentration of TNF was determined by enzyme linked immunosorbent assay (ELISA) and by bioassay. All samples were determined in duplicate. The concentration of IL-10 was determined by ELISA using a capture anti-IL-10 monoclonal antibody (JES3-9D7, Pharmingen, San Diego, CA, USA) and a biotinylated antibody for detection (JES3-1268, Pharmingen). IL-6 was determined by a sandwich ELISA obtained from Prof Aarden (Central Laboratory of the Blood Transfusion Services, Amsterdam, The Netherlands). IL-12 p40 subunit was determined by ELISA.

DATA ANALYSIS AND STATISTICS

Patients were analysed on the basis of completers. The correlation between the decrease in the amount of swollen joints and the decrease of TNF production capacity was calculated with the reduction in TNF production capacity (endotoxin induced TNF production on week 0 and week 16 divided by 2 minus endotoxin induced TNF production during treatment at week 4 and 8 divided by two) and the reduction of swollen joints

(number of swollen joints on week 0 and week 16 divided by 2 minus number of swollen joints on week 4 and week 8 divided by 2). Data were analysed with an analysis of variance (ANOVA) for repeated measures.

Results

TOXICITY OF PENTOXIFYLLINE/THALIDOMIDE

Twelve patients were enrolled in the study. The average age was 64.2 (SD 7.8) years and the disease duration 10.1 (5.4) years. All patients were rheumatoid factor positive and suffered from erosive disease. The number of disease modifying antirheumatic drugs previously used was 3.1 (1.5). The present medication was continued during the study and consisted of a disease modifying antirheumatic drug and a non-steroidal anti-inflammatory drug. Two patients also received low dose steroids. One patient developed a rash after one week of pentoxifylline/thalidomide which disappeared after the experimental medication was stopped. Two patients left the study because of severe constipation after one and four weeks, respectively. These patients were followed up on all scheduled visits. Side effects asked about from a pre-established list were xerostomia 33% (before treatment) and 100% (ever during treatment); drowsiness 33% and 77%; constipation 22% and 77%; oedema 44% and 66%; dizziness 11% and 33%.

TNF PRODUCTION CAPACITY AND PENTOXIFYLLINE/THALIDOMIDE

Whole blood cultures in the presence of endotoxin were performed to measure inhibition of production of TNF *ex vivo*. Whole blood cultures may yield variable results in one healthy person tested over time. Therefore one control person was tested on 24 different occasions. The mean amount of TNF produced upon culture with 100 ng endotoxin was 14.7 (SD 5.0) ng ml⁻¹ (range 7-23) and upon culture with 1000 ng endotoxin was 20.3 (5.9) ng ml⁻¹ (range 9-32). In order to detect changes despite this variability, blood was obtained before the medication, two times during medication, one day after cessation of medication, and four weeks after discontinuation of the medication. Table 1 shows that the amount of TNF produced was reduced significantly during treatment with pentoxifylline/thalidomide ($P = 0.01$, ANOVA). The amount of TNF produced in the whole blood cultures before the study (at week 0) was less than after the study but the change was not significant ($P = 0.33$). If TNF was measured by a bioassay instead of an ELISA, inhibition of TNF production was also found (ANOVA, $P = 0.037$) (table 1).

Table 2 Clinical variables. Baseline values are number of joints out of 28. Health assessment questionnaire (HAQ) has a maximum value of 3. Global assessments are normalised to 100%. Given is the percentage of reduction compared to baseline value for all patients (SD). P values are a Student's test between the differences before the course and after the course

Week	Before treatment	Immediately after treatment	4 weeks after cessation of treatment
Swollen joints	14.7 (3.9)	-30% (30%) (P=0.03)	-20% (40%) (P=0.15)
Tender joints	11.9 (6.5)	-40% (40%) (P=0.03)	-40% (30%) (P=0.01)
Restricted motion	17.8 (8.9)	-50% (20%) (P=0.003)	-30% (30%) (P=0.03)
Physician global assessment		-40% (40%) (P=0.04)	-60% (30%) (P=0.01)
"Grip strength"	18.5 (12)	+30% (40%) (P=0.2)	+60% (10%) (P=0.2)
HAQ	1.46 (0.7)	-10% (40%) (P=0.9)	-10% (70%) (P=0.9)
Patient global assessment		-20% (40%) (P=0.8)	-40% (40%) (P=0.1)
Morning stiffness		-10% (40%) (P=0.9)	-40% (40%) (P=0.3)
20% improvement criteria	0	44%	55%

Table 3 Changes in laboratory variables

	Week No					ANOVA test P
	0	4	8	12	16	
CRP	51 (36)	70 (27)	67 (43)	45 (38)	50 (37)	0.09
ESR	70 (32)	78 (30)	68 (30)	58 (33)	58 (32)	0.45
Hb	7.5 (1.1)	7.0 (1.1)	7.0 (1.1)	7.2 (1.1)	7.5 (1)	0.002
Albumin	43.6 (3)	41 (3.6)	40.5 (3.0)	45 (9.5)	43 (3)	0.22
Thrombocytes	412 (155)	470 (214)	420 (145)	411 (194)	372 (145)	0.05
Cortisol	0.39 (0.14)	0.49 (0.11)	0.48 (0.18)	0.41 (0.09)	0.38 (9)	0.4
Free cortisol	20 (9)	23 (5.6)	25 (12)	18.5 (5)	18 (10)	0.7
IL-6	11.8 (6.1)*	14.3 (12.6)	20.3 (30.3)	22 (36)	6.8 (4.4)	0.44
TNF	9.1 (17.5)	7.8 (16.6)	6.2 (11.3)	5.3 (7.8)	5.3 (9.5)	0.38

* = mean (SD).

To assess the specificity of pentoxifylline/thalidomide treatment for reducing TNF production, several other proinflammatory cytokines (IL-6, IL-12) and anti-inflammatory cytokines (IL-10) were measured in the whole blood culture supernatants. Table 1 shows that the production capacity for IL-6 was not affected, nor was the production of IL-12 or IL-10 (table 3).

ANTIRHEUMATIC EFFECTS OF THALIDOMIDE/PENTOXIFYLLINE

Of the nine patients who completed the study, five fulfilled the -20% improvement criteria of the ACR core set of outcome measures at the end of the treatment. If individual indices were analysed the swollen joint count, tender joint count, restricted motion, and disease assessment by the physician improved significantly (table 2). ESR and CRP did not change during treatment (table 3). Since the hypothesis guiding this trial was a putative relation between inhibition of production capacity of TNF and antiarthritic effects the correlation was tested between the clinical response and decrease in TNF production capacity. Such a correlation was not observed. Also no correlation was observed between the decrease in TNF production capacity and reduction in swollen joint count (correlation -0.57) tender joint count (correlation 0.8), restricted motion (correlation 0.18), grip strength (correlation 0.39), or disease assessment judgements (-0.76).

CHANGES IN ACUTE PHASE PROTEINS DURING PENTOXIFYLLINE/THALIDOMIDE TREATMENT

Table 3 shows laboratory variables during treatment. The trend in the laboratory variables was an increase in acute phase reactants. Some of the variables changed significantly, such as haemoglobin and thrombocytes, whereas others changed non-

significantly (C reactive protein, ESR, IL-6, and albumin). These changes might be caused by an effect of the medication, for example, on the liver or a direct effect on cytokine production. The IL-6 concentration in plasma was not significantly increased during treatment. No significant correlations were found between the changes in production capacity of IL-6, IL-10, or IL-12 and the differences in concentrations of acute phase proteins before and during treatment.

The hypothalamic-pituitary axis is independently activated by the inflammatory cytokines TNF α , IL-1, and IL-6.¹⁴ During pentoxifylline/thalidomide the cortisol levels were increased, although not significantly. Finally, TNF concentrations in plasma were measured. Table 3 shows that the amounts of TNF in plasma were low and variable. No significant changes could be measured during the course of the study.

Discussion

This study showed that the combination pentoxifylline and thalidomide is rather toxic. Symptoms related to side effects were observed in the majority of the patients. The incidence of side effects was similar to that in other studies except for a higher incidence of xerostomia.¹¹⁻¹³ The efficacy of simultaneous treatment with pentoxifylline and thalidomide in reducing TNF production was observed during treatment. This inhibition was specific, since production of IL-6, IL-10, and IL-12 was not affected.

The clinical effects of thalidomide/pentoxifylline were such that 55% of the patients reached the 20% improvement criterion. This could be due to a placebo effect, regression to the mean, or a real effect. The absence of a correlation between clinical effect

and the effect on TNF production does not suggest that the decrease in TNF producing capacity is causally related to reduction in disease activity, for example the swollen joint count. Moreover, the changes in the plasma IL-6, thrombocyte count, C reactive protein, ESR, albumin, haemoglobin, and cortisol were not suggestive of a reduction in TNF production in the arthritic joints. To clarify the issue of the clinical efficacy of pentoxifylline/thalidomide in rheumatoid arthritis a placebo controlled trial is necessary.

The relation between the reduction of endotoxin induced TNF production measured *ex vivo* and a possibly reduced TNF production in the arthritic joint or an antiarthritic effect is uncertain. In this study no such relations were found. In another study of pentoxifylline in rheumatoid arthritis, seven out of 16 patients improved but there was also no observed correlation between clinical improvement and reduction in TNF production.⁷ This lack of correlation can be explained by sample size or might suggest that inhibition in the endotoxin induced TNF production is not relevant for the inflammatory process in rheumatoid arthritis. This interpretation contrasts with the beneficial effects of phosphodiesterase inhibitors in murine models of arthritis in which inhibition of endotoxin induced TNF production occurred simultaneously with antiarthritic effects. In numerous clinical situations pentoxifylline is used to reduce TNF mediated effects. However, no data are available showing that a reduction in TNF production affects the production of acute phase proteins. This phase I study was initiated on the basis that continuous production of TNF in the arthritic joints could be inhibited in a similar way to the TNF produced in endotoxin stimulated whole blood cultures. However, the lack of association between reduction in TNF production in endotoxin stimulated whole blood cultures and laboratory variables reflecting joint inflammation or clinical improvement brings the value of this substitute index of pharmacological efficacy into question. A large trial in which clinical efficacy in rheumatoid arthritis is measured simultaneously with whole blood cultures should be performed to validate or

reject the use of whole blood cultures as a substitute index of pharmacological efficacy.

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