

Early onset and effective inhibition of bone resorption in patients with rheumatoid arthritis treated with the tumour necrosis factor alpha antibody infliximab

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Received on September 24, 2002; accepted in revised form on February 14, 2003.

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Key words: Rheumatoid arthritis, infliximab, bone resorption, TNF alpha.

Abstract

Objective. To investigate the effect of the tumour necrosis factor alpha antibody infliximab on bone metabolism in patients with rheumatoid arthritis (RA).

Methods. Twelve RA patients with active disease on a constant dose of methotrexate were treated with a single infusion of infliximab (10 mg/kg BW). Serum β -CrossLaps and serum osteocalcin as markers of bone resorption and formation were measured two days and one day before and one and 14 days after infliximab infusion with an electrochemiluminescence immunoassay. RA disease activity was determined using the Disease Activity Score (DAS) and the ACR-response criteria.

Results. Infliximab treatment significantly reduced serum β -CrossLaps levels from 0.29 ± 0.13 (mean \pm SD) ng/ml at study entry to 0.17 ± 0.09 pg/ml one day after infusion ($p < 0.005$). At day 14 serum β -CrossLaps levels were still significantly lower compared to pre-treatment levels (0.24 ± 0.13 pg/ml, $p < 0.05$). In contrast, serum osteocalcin levels remained unchanged during the observation period (17.8 ± 9.8 vs 18.2 ± 9.9 vs 18.6 ± 12.1 ng/ml, respectively). All but one patient improved clinically after infliximab infusion and the DAS dropped significantly from 6.5 ± 0.9 prior to treatment to 5.8 ± 1.3 and 5.0 ± 1.3 at Day one and 14 days after treatment, respectively. Four patients showed an ACR 20-response one day after therapy and 10 patients 14 days after therapy.

Conclusion. Infliximab might have potential to inhibit generalised bone loss in patients with RA in addition to its clinical efficacy in reducing disease activity and inhibiting joint destruction.

Introduction

Patients with rheumatoid arthritis (RA) are at increased risk of developing three different types of bone loss, i.e. joint erosion and juxta-articular or systemic osteoporosis. Bone loss is accompanied by vertebral fractures and hip fractures, further adding to their burden of invalidity (1). Glucocorticoid therapy, impaired mobility and, most importantly, disease activity associated

with high levels of circulating pro-inflammatory cytokines are linked to increased bone resorption and generalised osteoporosis in RA. Recent studies demonstrated that markers of bone resorption such as β -CrossLaps, which are released as fragments of Collagen type I during osteoclast driven bone resorption, are relevant predictors of annual bone loss in these patients (2, 3). Infliximab, a chimeric human-mouse monoclonal antibody that neutralises soluble and membrane bound TNF and is administered intravenously in combination with methotrexate, is highly effective in relieving signs and symptoms in patients with active RA and was shown to inhibit bone destruction especially at a dose of 10 mg/kg BW (4). Clinical improvement in infliximab treated patients was accompanied by a significant decrease in pro-inflammatory cytokine production such as interleukin-1 (IL-1) and interleukin-6 (IL-6) *in vivo* (5). *In vitro* studies suggest that both cytokines activate either directly or indirectly osteoclasts to trigger bone resorption (6), leading to the hypothesis that infliximab may exert a bone protective role in this disease.

Based on these observations we investigated the effect of infliximab on systemic bone turnover since the development of bone erosions in RA and the development of osteoporosis in individuals with high bone turnover are known to share several pathogenic mechanisms.

Patients and methods

Patients

Twelve RA patients (with moderate to severely active disease) classified according to the revised ACR-criteria (7 females and 5 males; mean age 56 ± 11 yrs; disease duration 13 ± 6 yrs) with normal renal function on a fixed dose of methotrexate (range 12.5 - 20 mg/wk) for at least one month, were enrolled in the study after giving informed consent (for details on patients see Table I). Eight of the 12 patients also received a constant dose of prednisolone equivalent to 10 mg or less per day for at least one month prior to and throughout the study. With the exception of corticos-

Table I. Demographic and medical characteristics of RA patients enrolled in the study and treated with a single infusion of infliximab (10 mg/kg BW).

Pt. no.	Gender	Age (yrs.)	Disease duration (yrs.)	Joint destruction Steinbrocker criteria	No. of prior DMARDs	Prednisolone equivalent (mg)
# 1	m	67	21.9	III	4	5.0
# 2	m	51	6.9	II	3	5.0
# 3	m	73	15.1	IV	6	5.0
# 4	m	58	15.1	IV	5	3.75
# 5	f	53	17.4	IV	5	10.0
# 6	m	57	12.9	III	3	5.0
# 7	f	51	11.9	III	5	0.0
# 8	f	58	22.9	III	5	2.5
# 9	f	37	5.1	II	6	0.0
# 10	f	67	11.9	III	3	0.0
# 11	f	39	11.5	IV	2	0.0
# 12	f	66	2.5	II	5	7.5

teroids, no other medication known to affect bone metabolism was prescribed during the entire study period. A single infliximab infusion was administered intravenously at a dose of 10 mg/kg BW according to the manufacturer's guidelines.

Methods

Blood samples were taken between 8 and 9 o'clock in the morning twice prior to infliximab therapy (i.e. Day -2 and -1) and one day (i.e. Day +1) and 14 days (i.e. Day +14) after treatment. Two consecutive blood samples were collected prior to initiation of therapy to preclude that circadian changes and intra-individual variation in any of the

parameters measured could account for differences between pre- and post-treatment values. Blood sera were frozen at -70°C until assay. Serum β -CrossLaps, a specific marker of bone resorption, and osteocalcin, an established marker of bone formation (7), were determined in duplicate by an electrochemiluminescence immunoassay (ECLIA) using the ELECSYS 2010 immunoassay analyser (Roche diagnostics GmbH, Mannheim, Germany). The sensitivity of the β -CrossLaps assay was 10 pg/ml with a range from 10 to 1000 pg/ml and the calculated intra-assay precision was 2.5%. The sensitivity of the osteocalcin assay was 0.5 ng/ml with a range from 0.5 to 300

ng/ml and the calculated intra-assay precision was 2.9%. C-reactive protein levels (CRP) were measured by nephelometry. No significant difference before infliximab treatment (i.e. between Day -2 and Day -1) was found for any parameter, indicating that intra-individual variation did not contribute to possible differences between pre- and post-treatment values.

Clinical assessment

RA disease activity was assessed twice before, and on Days 1 and 14 after infliximab treatment. The number of tender and swollen joints, the patient's global assessment (0-10), the physician's global assessment (0-10), the intensity of pain on a visual analogue scale (0-100), the Health Assessment Questionnaire (HAQ; 0-3) and C-reactive protein (CRP) (upper normal value 9 mg/l) levels were recorded and the Disease Activity Score (DAS) and the ACR20- and ACR50-Responses were calculated from the data.

Statistical analysis

If not indicated otherwise, all data are presented as mean \pm SD. Pre-treatment and post-treatment measurements were compared using the Wilcoxon Signed Rank Test; differences in the DAS before and after treatment were calculated with the two-tailed paired t-test. P values less than 0.05 were considered significant. Analysis was performed on a MacIntosh computer using the StatView 5.0 software package.

Results

Effect of infliximab on markers of bone metabolism

There was no significant difference in serum β -CrossLaps levels (mean coefficient of variation 45.3% vs. 50.5%) at two time points prior to infliximab therapy (Day -2; Day -1) and the average of the two measurements was used in the statistical analysis. Serum β -CrossLaps significantly decreased on average from 0.29 ± 0.13 to 0.17 ± 0.09 ng/ml one day after the infliximab infusion (Day +1) ($p < 0.005$) (Fig. 1), with a maximum decrease of 60% (patient #7), and a minimum decrease of 4% (patient #6). At day 14 serum β -Cross-

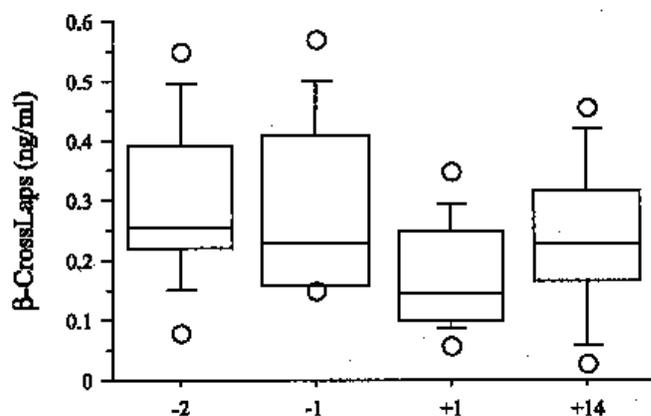


Fig. 1. Effect of a single infusion of infliximab (10 mg/kg BW) on serum β -CrossLaps levels measured two days (Day -2) and one (Day -1) day prior to treatment and one day (Day +1) and 14 days (Day +14) after treatment by an electrochemiluminescence immunoassay. The average of two measurements prior to infliximab therapy (Day -2, Day -1) was compared to post-treatment levels for the statistical analysis. * $p < 0.005$

Table II. Effect of a single infusion of infliximab (10 mg/kg BW) on the parameters of disease activity DAS and CRP and on serum osteocalcin levels as a marker of bone formation determined two days (Day -2) and one (Day -1) day prior to treatment and one day (Day +1) and 14 days (Day +14) after treatment. The average of two measurements prior to infliximab therapy (Day -2, Day -1) was used for statistical analysis of the DAS, CRP levels and osteocalcin levels. Data are presented as mean \pm SD.

	Pre-treatment		Post-treatment	
	Day -2	Day -1	Day +1	Day +14
DAS (0-10)	6.5 \pm 0.9	6.5 \pm 0.9	5.8 \pm 1.3*	5.0 \pm 1.3**
CRP (mg/l)	33.8 \pm 38.4	33.5 \pm 35.6	29.3 \pm 34.1*	13.0 \pm 16.1**
Osteocalcin (ng/ml)	17.1 \pm 9.9	18.4 \pm 9.9	18.2 \pm 9.9	18.6 \pm 12.1

DAS: Disease Activity Score; CRP: C-reactive protein. *P < 0.05; **P < 0.005

Laps levels were still significantly reduced compared to pre-treatment values, at 0.24 ± 0.13 pg/ml (Fig. 1) ($p < 0.05$), although -CrossLaps levels had started to rise again in 4 patients and reached pre-treatment levels in 2 patients with the smallest reduction in -CrossLaps at Day 1 after infliximab treatment (patients #6, #9). The reduction of -CrossLaps, however, remained stable up to Day 14 or declined even further in 5 patients. In 3 patients (patients #5, #11, #12) -CrossLaps could not be determined at day 14 because no blood samples were available.

Osteocalcin levels were 17.8 ± 9.8 ng/ml prior to treatment and remained at the same level at Day 1 and 14 days after infusion (18.2 ± 9.9 vs. 18.6 ± 12.1 ng/ml) (Table II). No significant correlation was found between the change in DAS as a marker of disease activity and of serum -CrossLaps at Day 1 and 14 days after the infliximab infusion (data not shown).

Clinical effect of infliximab treatment

All patients improved clinically after receiving infliximab. One day after infusion, 4 patients demonstrated a 20% ACR-response and 14 days after infusion, 10 patients experienced a 20% ACR-response and one patient a 50% ACR-response. The DAS improved significantly from 6.5 ± 0.9 prior to infusion to 5.8 ± 1.3 and 5.0 ± 1.3 one day and 14 days after treatment, respectively (Table II). In addition, CRP levels decreased from 33.6 ± 36.7 mg/l prior to treatment to 29.3 ± 34.1

mg/l one day after infusion and were significantly reduced to 13.0 ± 16.1 mg/l 14 days after treatment.

Discussion

Our open-label prospective study provides the first *in vivo* results that treatment of active, moderate to severe RA with infliximab may reduce bone resorption since serum -CrossLaps decreased significantly as early as one day after treatment. Release of -CrossLaps, known as fragments of bone-associated type I collagen, into the circulation seems to reflect bone resorption with high accuracy as demonstrated in recent studies in RA patients (8). In these studies -CrossLaps levels decreased significantly after hormone replacement therapy was started and increased when steroid therapy was initiated. We do not think that -CrossLaps levels simply represent another acute phase protein in RA since at the single patient level no correlation was found between improved disease activity and the reduction in -CrossLaps after infliximab therapy. For example, one patient (patient #3) experienced no improvement in the DAS but his -CrossLaps levels decreased by 55%. Evidence of at least partly independent regulation of bone resorption in RA patients treated with infliximab comes also from a placebo-controlled clinical trial, which showed that reduced radiological progression of disease could be demonstrated even in patients with minor clinical response to anti-TNF therapy (4).

Recently published studies support a

strong influence of TNF on bone resorption although there is still some controversy about the role of TNF in the regulation of osteoclast differentiation and activity. TNF may induce osteoclast differentiation and activation not only indirectly via osteoblast stimulation (9) but may also directly regulate osteoclast activity to cause bone resorption (10). TNF promotes differentiation of osteoclast progenitors and may increase bone resorption by stimulating the production of receptor activator of nuclear factor kappa B ligand (RANKL) (11, 12). TNF drives the production of the pro-inflammatory cytokines IL-1 and IL-6 considered to play a pivotal role in osteoclast stimulation (6). Elevated levels of TNF and of the soluble receptor for RANKL are detectable in blood sera in active RA, indicating that TNF may have a systemic effect on bone metabolism (13). Hence, blockade of TNF bioactivity with the neutralising TNF antibody infliximab may down-regulate osteoclast activity and bone resorption as demonstrated in our study. The significant decrease in disease activity within one day of treatment with infliximab may also have contributed to the reduction of bone resorption as recent studies have shown that bone loss in RA correlates well with cumulative disease activity (14).

Serum -CrossLaps levels rose again in 4 out of 12 patients as measured 14 days after infliximab therapy. However, pre-treatment levels were reached only in those 2 patients with the smallest decrease in -CrossLaps levels one day after infusion. Both patients had responded to infliximab therapy and the DAS fell by 37% and 14% respectively 14 days after treatment, implicating that the weak effect of infliximab on osteoclast activity in these 2 patients might have subsided earlier compared to its effect on inflammation. Although a clear dose response of infliximab on clinical and laboratory parameters of RA disease activity was found, these parameters may not change in parallel. In one of the first trials of infliximab in RA CRP levels started to rise again as early as one week after therapy, even in those patients receiving 10 mg/kg BW

of infliximab (15). The increase in serum CRP levels was independent of the number of swollen and tender joints, which continued to decrease and remained stable for up to 4 weeks after infusion. In another study infliximab led to a sharp fall in interleukin-6 serum levels one day after treatment, but serum levels increased again 7 and 14 days after infusion even at the high dose of 10 mg/kg BW (5). Both studies indicate that the inhibitory effects of infliximab on inflammatory and regulatory mediators may subside earlier than expected from the clinical data. Early loss of antiresorptive effects of infliximab on osteoclast activity may thus have contributed to the increase of serum γ -CrossLaps levels observed in some of our RA patients. It would of course have been interesting to measure γ -CrossLaps levels 7 days after infliximab infusion, but unfortunately we did not collect samples at that time point. Hirayama and co-workers could show that the bone-resorbing activity of peripheral blood mononuclear cell derived osteoclasts of RA patients is genuinely higher than that of healthy controls and that TNF had no influence on the number and activity of these osteoclasts (16). Therefore we assume that mechanisms independent of TNF may also be responsible for the increase in γ -CrossLaps levels in some of our patients 14 days after infliximab treatment. Finally, the mean serum half-life of infliximab varies from 9 to 12 days (17). It remains speculative if a difference in infliximab serum levels may have contributed to the different rate of bone resorption found 14 days after infusion, as we did not measure serum infliximab levels in our patients. Studies on a larger population of RA patients are warranted to gain more insight into the long-term effects of neutralising TNF activity on bone metabolism *in vivo*.

Osteocalcin is a major non-collagenous protein found in bone and teeth. Studies correlating osteocalcin serum levels with the rate of bone turnover, as assessed by iliac crest bone biopsy, indicate that it is a specific marker of

osteoblast activity (7,18). Since osteocalcin levels did not change after infliximab treatment, bone formation may either genuinely not be affected by the blockade of TNF activity or osteoblast activity and bone formation may change only many weeks after therapy is started. Support for a delayed effect on bone formation comes from a recent paper, in which reduction of bone formation was seen only several weeks after anti-resorptive therapy with bisphosphonates was initiated in postmenopausal women (19).

In conclusion, infliximab treatment of RA not only reduces clinical disease activity, as already demonstrated in recent trials, but may also ameliorate bone resorption, as shown by a significant reduction in serum γ -CrossLaps levels albeit in a small number of patients. Our preliminary findings raise the intriguing question as to whether infliximab suffices to prevent secondary osteoporosis in RA patients. Randomised controlled studies on a larger population of RA patients are warranted to further investigate the effects of repeated infliximab infusions on bone metabolism and especially on bone mineral density.

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