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Rapid improvement of bone metabolism after infliximab treatment in Crohn's disease

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SUMMARY

Background: Crohn's disease is associated with low bone mineral density and altered bone metabolism. *Aim*: To assess the evolution of bone metabolism in Crohn's disease patients treated with infliximab. *Methods:* We studied 71 Crohn's disease patients treated for the first time with infliximab for refractory Crohn's disease. Biochemical markers of bone formation (type-[procollagen N-terminal propeptide, bone-specific alkaline phosphatase, osteocalcin) and of bone resorption (C-telopeptide of type-I collagen) were measured in the serum before and 8 weeks after infliximab therapy and compared with values in a matched healthy control group.

Results: Eight weeks alter treatment with infliximab, a normalization of bone markers was observed with a median increase in formation markers of 14-51% according to marker and a lower but significant decrease in resorption marker (median 11%). A clinically relevant increase in bone formation markers was present in 50-61% of patients according to the marker. A clinically relevant decrease in C-telopeptide of type-I collagen was present in 38% of patients. No association was found with any tested demographic or clinical parameter.

Conclusion: infliximab therapy in Crohn's disease may rapidly influence bone metabolism by acting either on bone formation or bone resorption. This improvement seems to be independent of clinical response to infliximab.

INTRODUCTION

Crohn's disease (CD), a chronic inflammatory disorder of the gastrointestinal tract, has been associated with low bone mineral density (BMD).[1-6] According to their T-score. 54% and 78% of CD and ulcerative colitis patients were found osteopenic (T-score <-1) at the spine and at the femoral neck respectively.[7] while 1.8-42% and 29-41% of them were osteoporotic (T-score <-2.5) at the spine and at the femoral neck respectively.[8] More importantly, an increased risk of fracture was described in CD patients in several studies with a relative risk ranging between 1,32 and 2.5.[9-12] The largest risk was observed at the spine, particularly in women. with a b.5-fold increase in fracture risk.[9] Mechanisms potentially involved in bone loss in CD are multiple. encompassing malabsorption in general and vitamin D deficiency in particular.[13, l4] glucocorticoid treatment,[2, 15-17] hypogonadism,[6] vitamin K deficiency leading to undercarboxylation of osteocalcin (*OSC*),[18, 19] and the inflammatory process itself.[19, 20] The risk of fracture seems to be particularly associated with glucocorticoid treatment and disease activity.[12]

The CD patients with chronic active disease and either steroid dependency or resistance are usually good candidates for treatment with infliximab. a chimaeric monoclonal antibody against tumor necrosis factor alpha (TNFα).[21] Infliximab has proven to be very effective in both inducing and maintaining clinical remission in refractory CD patients.[22-25] Beyond this, infliximab therapy also induces a decrease in biological markers of inflammation.[22] mucosa! healing in a large number of cases[26] and favours steroid sparing.[27] Infliximab may therefore indirectly influence bone metabolism in CD. Furthermore, infliximab specifically blocks TNFα. which has a direct potent effect on bone remodelling by increasing bone resorption and inhibiting bone formation.[28-30]

The evaluation of the impact of a given treatment on bone loss includes several aspects: its effect on bone turnover, on BMD, and most importantly, its effect on the risk of new vertebral and non-vertebral fractures, the most relevant effect being obviously the last one. However, fracture risk assessment requires long-term prospective studies with a large cohort of patients. Such studies are difficult to perform and are not currently available in CD.[31] Changes in BMD occurring after an antiresorptive treatment have not been clearly associated with any fracture risk reduction.[32] In postmenopausal osteoporosis, it has been estimated that only 4-28% of the fracture risk reduction following treatment with antiresorptive drugs could be attributed to an increase in BMD,[33-35] Moreover. in CD and in gluco-corticoid-induced osteoporosis, a discordance between BMD and fracture risk has been described.[36] Biochemical markers of bone turnover have the advantage to be rapidly modifiable by treatment and to be independent predictors of the risk of osteoporotic fractures.[37] Indeed, recent studies have shown an association between change in markers of bone turnover and fracture risk reduction after raloxifene. risedronate and alendronate treatments.[38-40]

Our aim was to assess the short-term evolution of biochemical markers of bone turnover after a first infliximab treatment for active CD and to look for clinical or demographic factors associated with a positive response.

PATIENTS AND METHODS

Patients

Seventy-one patients with CD. included between November 1998 and August 2000 in an expanded *access* programme of infliximab in Belgium. were studied. All patients were treated with infliximab for the first time. To be included in the expanded access programme, patients had to be between 18 and 65 years of age, adopt adequate birth control measures, give informed consent, and have one of the following specific inclusion criteria: (i) a single or multiple perianal or enlerocutaneous draining fistula(e) as a complication of CD. resistant to conventional treatment for at least 3 months, (ii) moderate to severely active CD of at least 6 months duration, with colitis, ileitis or ileocolitis confirmed by radiography or endoscopy, refractory or dependent on oral steroid therapy (>8 mg/day prednisone equivalent) and/or non-responding to immunosuppressive agents (az-athioprine, mercaptopurine or methotrexate). Approval from the Ethics Committee was obtained in April 1998. Characteristics of the patients are shown in Table 1.

Table 1. *Characteristics of the patients*

Gender	2 3 M/48 F
Age (median, years)	36 (17-64)
Disease duration (median, years]	12 (1-30)
Smoking	30*
Disease location (Vienna classification)	11 L1. 26 L2. 29 L3. 5 L4
Steroid treatment	41
Immunosuppressive drug	37
Fislulizing disease	21
CDAI (median)	271 (154-610)
CRP (normalized. median)	4.4 (1-58)

* None of these smokers slopped smoking between infliximab treatment and bone markers measurements 8 weeks later. CDAI. Crohn's discasc activity index: CRP. C-reactive protein.

Control population

We also studied a healthy control population matched for age and gender with our inflammatory bowel disease (IBD) patients. This population included 23 males and 45 females, with a median age of 38 years (range: 22-76).

Infliximab treatment and biochemical markers of bone turnover measurements

All the patients had active disease [as defined by a CD activity index (CDAI) >150] before treatment and had never been treated with infliximab before. Patients were treated with a single infusion of 5 mg/kg infliximab at baseline for luminal refractory disease and with three infusions of 5 mg/kg infliximab at baseline, week 2 and week 6 for fistulizing refractory disease. Clinical activity of the disease was calculated by the CDAI before and 4 weeks after complete treatmenl (week 4 in luminal refractory disease, and week 10 in fistulizing refractory disease). A complete clinical response to infliximab was defined as a decrease of CDAI below 150. 4 weeks after

complete infliximab treatment.[22] A partial response to infliximab was defined as a drop of 100 points in CDAI. C-reactive protein (CRP) serum concentration was measured at baseline and 4 weeks after complete infliximab treatment to assess biological response to treatment. A positive biological response to treatment was defined as a 2 5% decrease in CRP level[41] and a complete biological response was defined as CRP normalization. Serum samples were also collected al baseline and 8 weeks alter complete infliximab treatment (week 8 for refractory luminal CD and week 14 for refractory fistulizing CD) for measurements of biochemical markers of bone turnover. Bone-specific alkaline phosphatase (BALP), OSC and type-1 procollagen N-terminal propeptide (P1NP) serum concentration were chosen as markers of bone formation markers and C-telopeptide of type-1 collagen (CTx) serum concentration as a marker of bone resorption. Levels were measured in the samples by using commercial kits according to manufacturer's instruction: BALP was measured using immunoradiometric assay (Tandem°-R Ostase from Beckman Coulter, Fullerton. CA. USA). OSC was measured by immunoradiometric assay (Osteo-RIACT from CIS Biointernational, Gif-sur-Yvette, France). P1NP was measured by radioimmunoassay (Intact P1NP RIA kit from Orion Diagnostica, Espoo, Finland), and CTx was measured by enzyme-linked immunosorbent assay (ELISA: Serum CrossLaps ELISA from Nordic Bioscience Diagnostics. Eerlev. Denmark).

Statistics

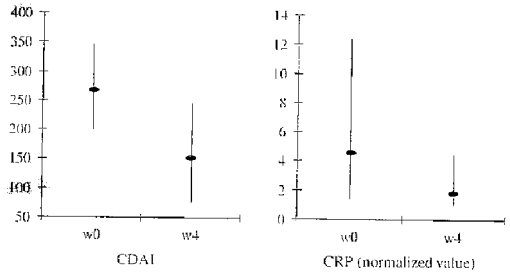
Serum concentrations of BALP, OSC, P1NP and CTx were compared at baseline and after 8 weeks of infliximab treatment by a paired non-parametric test (Wilcoxon). These values were also compared with values in a normal control population with non-parametric Mann-Whitney test. Correlation between evolution of these markers and evolution of CDAI or evolution of CRP were studied by Spearman non-parametric correlation test. Association was studied between a relevant change in bone formation or bone resorption marker and various demographic and clinical characteristics [age, gender, current smoking, disease duration, disease location, current steroid treatment. number of infliximab infusions (1 or 3), clinical response to infliximab, biological response to infliximab, steroid weaning], in univariate analysis and multivariate logistic procedure. A clinically relevant change in bone metabolism was defined as a 30% increase or decrease in BALP. OSC, P1NP or CTx serum concentration 8 weeks after infliximab treatment. These 30% thresholds were chosen based on the fact that antire-sorptive treatment is associated with a decrease in bone turnover markers by grossly 30-70% at 3-12 months.[38-40, 42, 43] These modifications are associated with significant changes in bone density or even fracture risk.[38-40, 42, 43] Level of significance was $P < 0.05$.

RESULTS

Clinical and biological response to infliximab in the cohort (evaluated 4 weeks after complete infliximab treatment)

A positive clinical response was observed in 75% of the patients 4 weeks after complete treatment, including 57.3% of complete response. Change in median CDAI is shown in Figure 1a. A positive biological response was observed in 66,7% of patients 4 weeks after treatment, including 28.6% of complete response. Change in median CRP is shown in Figure 1b.

Figure I. *Change in Crohn's disease activity index (CDAI) and C-rcactive protein (CRP), 4 weeks after infliximab treatment. Results are expressed as median and interquartile ranges. CRP values have been normalized according to the upper limit of normal range in each laboratory taking part in the study.*



Evolution of biochemical markers of bone turnover (evaluated 8 weeks after complete infliximab treatment)

Serum concentration of BALP, OSC and P1NP, markers of bone formation, were lower in CD before infliximab treatment than in controls while they return to normal levels alter infliximab treatment (Table 2). Serum concentration of CTx, a marker of bone resorption. was significantly increased in CD at baseline but was no longer different from controls after infliximab treatment (Table 2). The median relative change in BALP, DSC, P1NP and CTx concentrations 8 weeks after complete infliximab treatment are shown in Figure 2(a) and (b) for refractory luminal and fertilizing CD, respectively. There was no significant difference when comparing median changes in bone markers in luminal (treated with a single infliximab infusion) and iistuiizing CD (treated with three infliximab infusions). There was no significant correlation between the delta CDAI after infliximab and variations of any of the biochemical markers of bone turnover 8 weeks after complete treatment. There was no significant correlation between the delta serum CRP after infliximab and variations in any biochemical markers of bone turnover before and 8 weeks after complete treatment.

Figure 2*. (a) Relative changes in serum bone formation markers, bone-specific alkaline phosphatase (BALP). osteocalcin (OSC) and type-I procollagen N-leπninal propeptide (P1NP). as well as in the bone resorption marker C-telopepiide of lype-I collagen (CTx), after a single infliximab infusion in refractory luminal Crohn's disease (CD). These markers were measured, as described in the methods, in 50 CD patients with refractory luminal disease, just before and 8 weeks alter infliximab treatment. Percentages of change were calculated and are here expressed as the median and interquartile ranges, for each marker. (b) Relative changes in serum bone formation markers. BALP. OSC and P1NP. as well as in the hone resorption marker CTx after the completion of three infliximab infusions (baseline, week 2 and week 6) in refractory tistulizing CD. These markers were measured, as described in the Methods, in 21 CD patients with refractory iistuiizing disease, just before and 8 weeks after completion of infliximab treatment (14 week after first infusion), Percentages of change were calculated and are here expressed as the median and inlerquartile ranges, for each marker.*

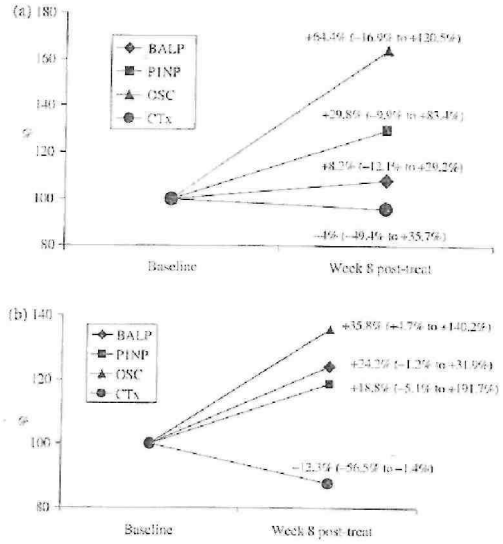


Table 2. *Serum concentrations of BALP. (OSC, P1NP and CTx in CD patients before and after infliximab treatment and in age and sex-matched healthy controls (median and interquartile range)*

	BALP (ng/mL)	**OSC (ng/mL)**	**P1NP (ng/mL)**	**CTx (pg/mL)**
CD before infliximab	7.5 (5.8-9.1)*	15 (8.7-18.6)**	30. 3 (20.2-40.6)***	256.6 (157-427.2)****
CD after infliximab	8.15 (6.2-12)	17.2 (12.8-28.3)	41.05 (27.7-61)	224.3 (103.1-374.1)
Healthy controls	8.l (6.5-10.5)	16.8 (12.7-21.5)	36.2 (30.8-55.3)	189.3 (137.8-248.1)

*P = 0.15 compared to controls (Mann Whitney) and P = 0.0008 compared to CD after infliximab (Wilcoxon).
** P = 0.04 as compared to controls (Mann Whitney) and P = 0.001 as compared to CD after infliximab (Wilcoxon).
*** P = 0.02 as compared to controls (Mann Whitney) and P = 0.003 as compared to CD after infliximab (Wilcoxon).
****P = 0.0055 compared to controls (Mann Whitney) and P = 0.04 as compared to CD after infliximab (Wilcoxon).
CD. Crohn's disease: BALP, bone-specific alkaline phosphatase; OSC. osteocalcin: P1NP. type-I procollagen, N-terminal propeptide: CTx. C-telopeptide of type-I collagen.

Relevant improvement in bone formation and resorption markers

A predefined relevant improvement in bone formation (increase of at least 30% in the bone formation marker) was found after infliximab treatment in 29.7%, 60.8% and 46.5% of the patients when considering BALP, OSC or P1NP as marker. respectively. The relative changes in these markers were significantly correlated to each other ($r = 0.58$, $P < 0.0001$; $r = 0.51$, $P = 0{,}0003$; $r = 0.80$, $P < 0.0001$, for correlations between BALP-P1NP, BALP-OSC, and OSC-P1NP. respectively). No significant association was found between the 30% increase in BALP, OSC or P1NP and any of the clinical or demographic parameters tested in univariate or multivariate analysis, including clinical or biological response to infliximab. A predefined relevant improvement in bone resorption (decrease of at least 30% in CTx serum levels was found after infliximab treatment in 38.2%. No significant association was found with any of the clinical or demographic parameters tested in univariate or multivariate analysts, including clinical or biological response to infliximab. A relevant-increase in at least two bone formation markers and/ or a relevant decrease in the bone resorption marker were present in 59.2% (42 of 71) of the patients. Despite an apparent uncoupling of bone metabolism (decreased bone resorption associated with increased bone formation) after infliximab when looking to the study population as a whole, we observed this uncoupling on individual basis, in only 8.5% (six of 71) (defined by a decrease of at least 30% in bone resorption marker and an increase of at least 30% in at least two bone formation markers).

DISCUSSION

Our data show a significant and rapid normalization in the biochemical markers of bone turnover in CD patients treated with infliximab. This improvement is characterized by either an increase in bone formation or a decrease in bone resorption and may probably be considered as clinically relevant in approximately 60% of the patients. This improvement does not seem to be selectively associated with demographic or clinical characteristics of the patients. including clinical or biological response to infliximab or even steroid weaning. The CD has been associated with altered bone metabolism and in particular, with an increase in bone resorption markers without a compensatory increase in bone formation markers during the active phase of the disease[7, 8, 13, 44] and with decreased bone formation associated with normal bone resorption in quiescent disease.[45] This profile may become even worse during systemic steroid treatment[46, 47] while no significant change is detected with budesonide.[46] Several treatments have been evaluated on the maintenance of bone mass in CD. Calcium and vitamin D supplementation or sodium fluoride were able to appreciably increase BMD over a 1-year period.[48-50] Alendronate significantly increased BMD in CD patients after J year of treatment.[42] In this study, biochemical markers of bone turnover (including OSC) decreased by amean of 30-40% after 6-12 months. Our data show that infliximab treatment may have an effect on bone metabolism which is radically different from other acute treatments of CD. particularly steroids, and may even have a beneficial impact. quantitatively comparable with the one reported for drugs specifically given to improve bone metabolism in CD. The effect is, however, qualitatively different from the one observed with alendronate. which is essentially an antiresorptive drug. With this drug both formation and resorption markers decreased over 6 months of treatment in CD.[42] With parathyroid hormone, which has never been tested in CD, an increase in bone turnover is expected with a coupled increase in bone formation first and secondly, in bone resorption.[51] The profile observed here with infliximab is atypical and may be more complex. Indeed, we observed an apparent uncoupling of bone metabolism associating a prominent increase in bone formation (increase of BALP, P1NP and OSC of a magnitude of 14-51%) with a tower but still statistically significant decrease in bone resorption (around 10%). Thisis comparable with what is observed with strontium ranelate, a drug for postmenopausal osteoporosis that dissociates bone remodelling with a 8% increase in BALP and a 12% decrease in serum CTx at 3 months.[52] However, when looking at individual patients. we found patients with either a significant increase in bone formation or a decrease in bone resorption and only a small minority of the patients had actually uncoupling evolution of bone markers. The effect of infliximab on bone metabolism in CD seems thus to be heterogeneous. with mainly an increase in bone formation in some patients while others have predominantly a decrease in bone resorption. This heterogeneity in bone response may be linked to the heterogeneity of the population studied on one hand and to the potentially multiple mechanisms of action of infliximab on bone metabolism, on the other hand. Considering the heterogeneity of the study population, there were differences in gender, age, smoking habit, steroid treatment that may certainly influence the bone response. However, neither a significant decrease in bone resorption nor a significant increase in bone formation was associated with any of these clinical and demographic factors, As far as the various possible mechanisms of action of infliximab, a first hypothesis is that it could act indirectly by diminishing gut inflammation. Nevertheless, the univariate or multivariate analysis found no significant association between either a significant decrease in bone resorption or increase in bone formation and the clinical or the biological response of the intestinal disease to the drug. Another possible explanation is a direct effect of TNFα blockade in the bone microenvi-ronment. TNFα is a known inducer of osteoclast differentiation by mechanisms that might be at least independent of the receptor activator of NFκB (RANK)/ RANK ligand pathway.[29, 53] Moreover, TNFα

inhibits osteoblast differentiation possibly by down-regulating a key osteoblast differentiating nuclear factor RUNX2-cbfal.[28, 54] Alternatively, other mechanisms depending on infliximab but not directly related to CD clinical or biological activity may be involved but remain to be elucidated. At this stage, it is difficult to determine the clinical significance of our results. It is important to note, however, that after infliximab treatment, the serum concentration of the various bone metabolism markers was no longer different from the one of age- and sex-matched healthy controls. We further judged the changes in bone metabolism as relevant when the increase in BALP, OSC or P1NP, or the decrease in CTx was >30%. The level of 30% was chosen by analogy to the results observed with alendronate in CD where a mean decrease of 30-50% was observed after 6 months in the treatment group while the mean change in the placebo group was <5%.[42] Furthermore, in this alendronate study, this magnitude of change in bone metabolism markers was associated with a significant improvement in BMD. Similar changes have been reported in postmenopausal women treated for osteoporosis with antiresorptive drugs. Interestingly, median changes of 52-41% at 1 year from baseline in bone formation markers in raloxifene-treated women were associated with a risk reduction for new vertebral fractures at 3 years,[43] Moreover, a recent study in patient treated with risedronate demonstrates thai there was no further benefits in facture risk with further decrease in bone resorption below the level of 35-60%. Among our infliximab-treated patients, a large majority (around 60%) had either a significant increase in bone formation or a significant decrease in bone resorption. The long-term effect of infliximab should ideally be assessed on the risk of fracture, which is the most relevant end-point. While there are currently no data on fracture risk reduction in CD patient after infliximab therapy, there are preliminary data showing an impact of this drug on BMD. In a pilot study of 13 patients, a significant increase in hip and spine BMD was reported 6 months after the first infliximab infusion,[55] suggesting that BMD changes may be associated with the biochemical modifications we have observed in our study.

In conclusion, infliximab induces a rapid improvement in biochemical markers of bone turnover, which is characterized by either increased bone formation or decreased bone resorption. The mechanism of action of infiximab on bone metabolism as well as the long-term effects on fracture risk remain to be determined.

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