Special Article

Disease-modifying antirheumatic drugs and bone mass in rheumatoid arthritis

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Received on February 2, 2005; accepted on February 15, 2005.

Clin Exp Rheumatol 2005; 23: 137-144.

Key words: DMARDs, bone metabolism, bone mineral density, rheumatoid arthritis, spondyloarthropathies, methotrexate, cyclosporine, leflunomide, biologic agents.

ABSTRACT

This article reviews the effects of DMARDs (including biologic agents) on bone metabolism in rheumatoid arthritis (RA). At present there is no evidence that methotrexate, at least at dosages ranging from 5 to 20 mg/week, negatively affects bone mass as measured by DXA (BMD) as documented in both cross-sectional and longitudinal studies. Most studies of cyclosporine (CyA) use reporting a reduction in erosions and joint damage with no adverse effects on bone, did not measure BMD; CyA treatment is associated with a dose-dependent increase of bone turnover as well as a decrease in both animal and human studies; however, its use in RA setting at a dose ≤ 5 mg/Kg/day has so far not been associated with clinical relevant adverse effects on bone metabolism. Anti-TNF-α agents, infliximab reduced markers of bone turnover in two longitudinal studies. Data on BMD are not available in RA; nevertheless, an increase in BMD has been documented in spondyloarthropathies with infliximab and etanercept. No clinical data concerning BMD are available on leflunomide as well as on the newer biologic agents (adalimumab, rituximab, anakinra).

Introduction

Over the past decade the therapeutic approach to patients with rheumatoid arthritis (RA) has shifted towards the earlier and more aggressive use of disease modifying antirheumatic drugs (DMARDs) (1, 2). In fact, scrupulous examination of the natural history of RA has demonstrated that 90% of those patients who develop erosions did so within the first 2 years of the disease course (3), as detected by currently available imaging techniques such as MR.

This aggressive approach to the management of RA is based upon the availability of new, highly effective DMARDs including cyclosporin A (CyA), methotrexate (MTX), leflunomide and biologic agents such as anti-TNF-α (etanercept and infliximab). These drugs can be used either as monotherapy or in combination (4). As a result the conventional therapeutic "pyramid" has been inverted (5) and reconstructed, the goal now being to prevent joint damage in early disease, prior to the development of extensive long-term damage (6, 7).

Generalized bone loss (8, 9) and an increased rate of vertebral (10, 11) and hip fractures (12, 13) have been well documented in the past, as well as in recent papers including a large cohort of RA patients (14-17). The pathogenesis of these processes is multi-factorial, involving both disease-specific [disease activity, cytokines, reduced physical activity and glucocorticoid (GC) use] and non-disease-specific factors (age, female sex, postmenopausal status) (18, 19). Additional potential risk factors for bone loss in RA could include treatment with DMARDs such as MTX and CyA, both of which have been associated with the development of osteoporosis in animal models. In fact recent studies suggest that these and other immunosuppressive agents may cause generalized osteoporosis in humans, as well. This article will critically review the possible effect on bone mass in RA patients of MTX and CyA, and in addition will highlight the possible effects on bone metabolism of leflunomide, anti-TNF-α drugs and other biologic agents.

Methotrexate

MTX, a folic acid antagonist, emerged as an "anchor drug" for the treatment of RA during the 1990s (20), but it is also a chemotherapeutic agent commonly used in patients with malignant disease. The triad for MTX osteopathy includes osseous pain, osteoporosis and stress fractures localised to the distal tibiae and was first described in children with leukemia who were receiving low-dose long-term maintenance therapy; these clinical signs reversed after discontinuation of MTX (21). Further similar cases have since been reported (22-24). The competitive inhibition of dihydrofolate reductase, an enzyme that converts dihydrofolate to tetrahydrofolate, is crucial for DNA biosynthesis and purine synthesis, and has been proposed as a mechanism of MTX osteopathy. In human bone-derived osteoblasts (25, 26) MTX showed a dose-dependent inhibition of osteoblast proliferation. This was confirmed in animal models: MTX (at levels equivalent to the standard dose used in RA patients) given weekly to rats for a 16-week period significantly reduced bone mass by decreasing bone formation and increasing bone resorption (27).

A systemic effect in humans seems to be unlikely since Bologna *et al*. (28) found that concentrations of MTX in synovial and bone samples from RA patients approximately 24 hours after a single 10 mg dose administered intramuscularly were 10-fold higher than concomitant plasma values.

Twenty years after the first reports of Ragab (21) and O'Regan (22), Preston *et al*. described in 1993 two patients, one with psoriasis and the other with RA, who developed clinical features of MTX osteopathy after long-term treatment with low-dose MTX (29). Other cases of non-oncologic patients with stress fractures associated with MTX have been subsequently reported and the role of MTX as a causal agent has been proposed. However, most of the 11 patients thus far described in the literature concurrently presented other well-known risk factors for fractures such as previous vertebral fractures and low plasma levels of vitamin D3 (30), long-term GC treatment and/or joint deformities (27, 31-33). The same risk factors were present in 8 of the 13 RA patients described by Alonso-Bartolomè *et al*. who developed stress fractures following MTX therapy (34).

The actual role played by MTX in reducing bone mass and thus predisposing to fractures in rheumatic patients remains controversial. In rats with adjuvant-induced arthritis, MTX maintains bone mass by preventing both a decrease in bone formation and an increase in bone resorption in a dose-dependent manner (35). These data have been confirmed by others in arthritic animal models (36) and have also been observed in RA patients. El Mediany *et al*. (37) showed that a weekly dose of MTX 10-15 mg significantly increased bone alkaline phosphatase and reduced deoxy-pyridinoline (DPYR), a marker of bone resorption, after 9 months in 30 female RA patients. More recently, in a prospective longitudinal study of 117 RA patients (90 F, 27 M) starting or continuing long-term treatment with MTX, no adverse effects on bone turnover markers were seen after MTX treatment (38). In this study, which represents the first histomorphometric report on the effect of low-dose MTX, the indices of bone formation in biopsies taken before and after MTX treatment in 4 subjects were not negatively influenced by the treatment.

In both animals and humans the effects on bone markers were associated with a significant improvement in inflammatory signs and in disease activity. Thus, MTX may exert a protective effect on bone metabolism by controlling disease activity. Confirmation of this hypothesis seems to have been provided by densitometric studies in RA patients. Buckley *et al*. in 1997 (39) reported data on bone mass density (BMD) measured by dual emission X-ray absorptiometry (DXA) from a prospective, randomised, placebo-controlled trial on the effect of prednisone therapy in patients receiving different DMARDs including MTX. BMD was measured at the lumbar spine and femoral neck at baseline and then annually for 3 years. Among patients who did not receive prednisone, at the end of the study there were no significant differences in BMD between those treated with MTX and those receiving other DMARDs. However, among patients on treatment with prednisone ≥ 5 mg/ day, MTX therapy was associated with bone losses at the lumbar spine greater than those observed in the patients receiving prednisone plus other DMARDs. This seems to suggest that MTX has a deleterious effect on trabecular bone only in prednisone-treated patients, by compounding the inhibitory effects of prednisone on bone formation. Another study (40) failed to detected significant differences in BMD measured at the lumbar spine and the femoral neck in 2 groups of post-menopausal RA patients, 10 of whom were treated with MTX for more than 3 years and 10 of whom did not receive MTX; however, the results were not corrected for age, which was significantly higher in patients not receiving MTX.

Our group reported the results of a 2-year longitudinal study carried out on 62 female RA patients treated with MTX (group A, n = 32) or other DMARDs (group B, n = 30) (41) and maintained on a low (≤ 7.5 mg of prednisone) constant GC therapy. The primary outcome of the study was a change in lumbar BMD every 12 months during treatment with MTX or other DMARDs. After 2 years both groups showed a comparable, significant reduction in BMD. When the patients were subdivided according to the severity of the disease those with more active disease had significantly greater BMD loss than those with less active disease, independently of the DMARD given.

Minaur *et al*. suggested that disease activity may act as a confounding factor in the BMD loss seen after MTX treatment. In a one-year prospective longitudinal study (38), MTX treatment was associated with lower BMD Z-scores, which were significantly different at the femoral neck then those observed with other DMARDs. However, by multivariate covariance analysis it was shown that the observed reduced Z-score levels were actually due to confounding factors such as disease severity and activity. The authors concluded that low-dose MTX had no adverse effect on bone density in patients with

RA. A recent cross-sectional study (42) which compared 30 patients treated with MTX (10 mg/weekly for a mean of 6 years) to 30 RA patients who had never received MTX, detected no adverse effect of MTX in spite of the concurrent higher daily dose of GCs in these patients.

The effect of low-dose MTX on BMD was recently evaluated in a large multicenter cross-sectional study involving 731 female patients with RA (43). The patients were selected from a large multicenter cross-sectional study on the frequency of osteoporosis and the main determinants of BMD in 925 female patients with RA (14). They were subdivided in those who had never taken MTX (n = 485) and those who had been taking MTX for at least 6 months (n = 246). The frequency of osteoporosis (defined as a T-score that was ≤ -2.5) between the two groups was 29.1% and 28.3% (p = NS) at the lumbar spine and 34.8% and 37.8% (p = NS) at the femoral neck, respectively. Mean T-score values at the lumbar spine and femoral neck were comparable in the two groups even after adjusting for age, menopausal status, BMI, Health Assessment Questionnaire (HAQ) score and GC use. The generalised linear model showed that age, menopause status, BMI, HAQ score and GCs were significant independent predictors of BMD, whereas MTX use was not. Moreover, the multivariate analysis showed that only age, HAQ score and BMI were significantly associated with the risk of osteoporosis (43).

We conclude that a large body of recent data contradicts the hypothesis that low-dose MTX negatively affects bone density and bone turnover in RA patients. It appears more likely that MTX can exert a protective effect on bone mass and bone turnover by interfering with both the production and activity of some inflammatory cytokines, specifically IL1 and TNF-α (44, 45), which are potent stimulators of osteoclastic bone resorption (46, 47).

Some points, however, remain still unresolved. For example, it is conceivable that in patients with inactive RA the potential osteopenic effect of MTX may become visible since it is no longer counterbalanced by its – much more important – effect on disease activity. The deleterious effect of MTX in diseases in which there is no bone mass involvement *per se*, such as polymyositis and polymyalgia rheumatica, might be completely different. It also remains to be clarified whether folinic acid supplementation may prevent bone loss in MTX-treated patients, since it has been shown to prevent MTX-induced toxicity in osteoblasts-like cells *in vitro* (48). Lastly, could the higher dosages that are being more and more widely used (25-30 mg/week) have a more deleterious effect on bone mass?

Cyclosporin A

The discovery in the 1980s of the powerful immunosuppressive actions of the fungal cyclic peptide CyA has revolutionized transplantation medicine. CyA inhibits the activation of T lymphocytes and thus graft rejection, mainly via transcriptional suppression of the interleukin-2 gene (49, 50).

Soon after, CyA was also widely studied for the treatment of various immunological and rheumatic diseases, but generally in lower doses than those used after organ transplantation. CyA has proved to be effective in both advanced and early RA and it is now registered in most countries for the treatment of the disease (51-54). At the doses commonly used for the treatment of RA the toxicity is considered manageable, although some caution should be exercised when administering it to patients with renal impairment and hypertension (55). In a number of studies it has been suggested that radiological progression is retarded by CyA in comparison with placebo or other DMARDs (56, 57). The combination of MTX with CyA has been shown to offer complementary efficacy, because the two drugs have different mechanisms of action and their toxicity patterns do not overlap (58).

A common and serious side effect of allogeneic organ transplantation is osteoporosis and related fractures and CyA may contribute to this pathogenesis (59). However, in most transplant recipients CyA is co-administered with other immunosuppressive drugs which are known to adversely affect bone, such as GCs (60), making it difficult to address the question of the skeletal effects of CyA in clinical studies. Some studies have indicated a deleterious effect of CyA on bone mass (61-63), whereas other clinical trials have suggested that monotherapy with CyA may not be associated with bone loss (64-68). Moreover, a study in kidney transplant patients reported that CyA may actually counterbalance the adverse effects of GCs on the skeleton (69). Thus, the risk of osteoporosis in transplantation as a result of treatment with CyA remains controversial.

CyA in rheumatic diseases is used at doses that do not exceed 5 mg/kg/day (51, 70) and at these doses it has not been associated with significant concerns about increased fractures. In a number of studies CyA treatment was actually associated with a reduction in the radiological progression of RA (56, 71-72) without apparent bone-related toxicity, although BMD was not measured in any of these studies. The only study specifically addressing bone metabolism in CyA was carried out by Ferraccioli *et al*. (73) in patients with early erosive, aggressive RA and a poor previous response to treatment with MTX. In these patients an average BMD decline of 4% occurred during the first 6 months of MTX treatment. After adding CyA at a dose of 3 mg/kg daily for 6 months, clinical variables and acute phase reactants improved significantly and BMD increased by 3.9%.

In murine models, CyA as well as other calcineurin inhibitors (tacrolimus or FK506), cause high turnover osteoporosis (59). CyA stimulates both osteoclast and osteoblast activity *in vivo*, but resorption rates exceed formation rates, with a net loss in bone mass (74, 75). A major side effect of CyA therapy is dose-related, acute and chronic nephrotoxicity, often leading to secondary hyperparathyroidism, which may also adversely affect skeletal health. Interestingly, CyA-induced osteopenia is attenuated by parathyroidectomy (76). Tacrolimus (FK-506), a fungal macrolide, also induces severe trabecular bone loss in rats (59), although this bone loss

may be less severe in humans compared to that induced by CyA (77).
At present, the molecular and cellular mechanisms for the skeletal effects of CyA are still unknown. It has been reported that CyA-induced bone loss does not occur in T-lymphocyte-deficient male nude rats (78), strongly suggesting a role for T lymphocytes or other immune cells in the pathogenesis of CyA-induced osteopenia.
It has been shown that CyA is anti-resorptive and bone-sparing in aged female rats but increases bone resorption and reduces bone mass in aged male rats (79). However, even in male rats CyA treatment, at clinically relevant doses, increased bone resorption only transiently and did not result in pronounced long-term cancellous bone loss. In this context, it is interesting to note that the inhibitory effects of CyA in whole blood lymphocyte proliferation assays show gender-related differences in rats and humans (80). Currently, it is not known whether the skeletal response to CyA may be different in women and men. A prospective clinical trial conducted over a period of 18 months in renal transplant recipients reported an increase in lumbar spine BMD in comparison to baseline values in response to CyA monotherapy, which was significantly greater in women compared with men (66).
It has been shown that CyA and FK-506 can decrease osteoprotegerin (OPG) mRNA by 65% and increase RANKL (receptor activator of nuclear factor κB ligand) mRNA levels by 120% in undifferentiated marrow stromal cells (81). These two actions are associated *in vivo* with the increased recruitment and activation of osteoclasts (82, 83). OPG-deficient mice develop both osteoporosis and arterial calcification, In the same study it was shown that CyA suppresses OPG production in coronary artery smooth muscle cells. Taken together these findings suggest a potential mechanism for immunosuppressant-induced bone loss, and the propensity of CyA to cause vascular disease.
In summary, CyA therapy has been shown to increase bone turnover and this effect is not associated with an equivalent increase in the bone formation rate. The resulting uncoupling of resorption over formation is likely to be mediated by T cells and the RANK/RANKL pathways and may cause bone loss and, possibly, an increased risk of fracture post-transplantation. However, this potential deleterious effect of CyA therapy is counterbalanced in the clinical setting by its GC sparing effect and by the suppression of the inflammatory process, particularly in rheumatic diseases, where the doses do not exceed 5 mg/kg/day.

Leflunomide

Leflunomide inhibits *de novo* pyrimidine biosynthesis by acting on dihydroorotate dehydrogenase (DHODH) (84-85) and this is associated with the suppression of proliferation and activation of T cells (86). It has been introduced as an immunosuppressive drug in patients undergoing the transplantation of allografts (87) and more recently for the treatment of RA (84). In patients with RA and in animal models of the disease it was shown to prevent joint bone erosions (88, 89). This action is, at least in part, independent of T cells. There is some evidence that leflunomide acts directly on the osteoclast precursor cells of the monocyte/macrophage lineage (90, 91).
Thus, leflunomide has the potential to prevent both focal and generalized bone loss by directly inhibiting osteoclastogenesis and osteoclast function. At the moment however, there is no significant clinical data to support this positive effect of the drug.

Anti-TNF-α agents

RA is characterised by the presence of an inflammatory synovitis accompanied by the destruction of joint cartilage and bone. Generalised osteoporosis is also a well-known phenomenon in RA, as demonstrated by decreased BMD in several studies (92). The cause of this generalized bone loss is multifactorial and still poorly understood, but changes in circulating levels of cytokines, e.g. IL1 and TNF-α, are definitely implicated. The overproduction of these two cytokines is pivotal in the pathogenesis of the joint inflammation and damage (93).
Osteoclastic bone resorption has been implicated in the pathogenesis of joint erosions in RA (94). Osteoclasts form from circulating monocytic precursors in the presence of the key signal RANKL (95). Expression of RANKL has been demonstrated in rheumatoid synovial T cells and fibroblasts (96, 97). However, as T cell expression of RANKL occurs on activation in most inflammatory settings, it is likely that the osteoclast formation in RA is a consequence of a highly osteoclastogenic environment which involves interactions between RANKL and other (inflammatory) signals, and between the monocytic precursors of the osteoclasts and other cell types. These cytokines act indirectly by up-regulating the osteoblast expression of RANKL (98). A direct effect of TNF-α on osteoclast precursors has also been suggested (99) but this effect is critically dependent upon IL1 (100) and the synthesis of prostaglandin E2. Clinical trials with TNF-α and IL1 inhibitors (101-103) indicate that clinical improvement and the halting of radiographic progression occur earlier than with conventional DMARDs.
The generalized bone loss in RA, but also in ankylosing spondylitis (AS), is related to biochemical markers of bone resorption on the one hand, and to markers of disease activity such as the erythrocyte sedimentation rate, serum C reactive protein and TNF-α circulating levels, on the other (104). This cytokine has been shown to mediate the increase of bone resorption both in the systemic osteoporosis related to oestrogen deficiency (105), and in periarticular or periprosthetic bone erosions. In a model of transgenic mice expressing soluble TNF receptor to neutralise TNF-α, animals were protected from oestrogen deficiency-related bone loss (106). TNF-α is also a powerful inhibitor of bone formation (107).
Taken together, these findings predict that the inhibition of TNF-α and IL1 might prevent both joint bone destruction and generalized bone loss. Unfortunately, data on BMD during treatment with anti-TNF-α agents in RA patients is scarce. A recent study documented a reduction in the urinary ex-

cretion of pyridinoline (PYR) and DPYR, both of which are markers of bone resorption, during 9 months of therapy with infliximab in 17 RA patients; the changes in bone resorption markers mirrored the clinical variables and acute phase reactants, which improved significantly (108). Changes in the markers of bone metabolism were also evaluated in 22 RA patients treated with infliximab for 46 weeks; a significant decrease in bone markers, associated with a fall in the serum levels of acute phase proteins, was seen at weeks 30 and 46 (109).

Some recent uncontrolled longitudinal studies have documented a positive effect in spine and hip BMD as well as in bone resorption markers in patients with AS and related spondyloarthopathies (SpAs) who were treated with infliximab (110), etanercept (111) or both (112).

This effect of anti-TNF-α treatment has also been observed in patients with Crohn's disease, in whom therapy with infliximab (5 mg/kg) caused a significant increase in the markers of bone formation (serum bone-specific alkaline phosphatase and total osteocalcin), but no significant change in the bone resorption marker serum N-telopeptide crosslinked type 1 (113).

In summary, the effect of anti-TNF-α treatment on bone metabolism has not yet been thoroughly investigated. Preliminary finding seem to predict a potential beneficial effect linked to increased bone formation.

Other biologic agents

No data are currently available regarding the effect on BMD and bone metabolism of the newer anti-TNF-α (adalimumab, rituximab), anti-IL1 (anakinra).

Conclusions

Generalised osteoporosis associated with an increased rate of fractures has been widely documented in RA. The pathogenesis of this process has been linked to various disease- and non-disease-related factors, and highly effective DMARDs such as MTX and CyA are considered to count among these risk factors. Concern has been raised among rheumatologists at the occurrence of osteoporosis and fractures in animal models, in patients receiving MTX for cancer or rheumatic conditions, and in organ-transplant recipients taking CyA.

Table I summarizes the data presented in this review on the effect on bone mass of the DMARDs most commonly used in RA. Regarding MTX, many longitudinal and cross-sectional studies carried out on RA patients failed to document a negative effect on BMD, at least at the low dosages commonly used (10-20 mg/week). No sufficient clinical data are available regarding the effect on bone mass of CyA and the only study in RA documented an increase of BMD after 6 months of treatment. However, *in vitro* and animal studies showing that CyA causes increased bone turnover with uncoupling of resorption over formation, as well as clinical data indicating a possible role of CyA in transplantation-related osteoporosis, suggest a dose-related effect, which may nevertheless be insignificant at the low dosages (3-5 mg/Kg/day) recommended for RA. There is some *in vitro* data that leflunomide directly inhibits osteoclasts, but to date there is no clinical evidence that this agent may have a positive effect on bone mass. Finally, the pivotal role of cytokines, e.g. IL1 and TNF-α, in the pathogenesis of systemic osteoporosis as well as in the bone erosions of RA suggests a possible beneficial effect on bone mass of anti-TNF-α agents and other biologic drugs. However, while two studies have been published on patients with SpAs, at present clinical data on BMD in RA are still lacking.

Table I. DMARDs and bone mass in RA patients.

MTX	• No adverse effect on BMD in prospective, longitudinal studies (38-41) as well as in cross-sectional studies (42, 43) • MTX mean weekly dose: 10 mg; range: 7.5-20 mg • Treatment duration: 1-6 years
CyA	• Increased BMD in an open longitudinal study (73) • CyA dose: 3 mg/Kg/day • Treatment duration: 6 months
Leflunomide	• No clinical studies on BMD • Inhibitory effect on osteoclastogenesis in *in vitro* studies (90, 91)
Anti-TNF-α agents	• No clinical studies on BMD • Significant reduction of markers of bone turnover in longitudinal studies with infliximab (108, 109) • Treatment duration: 9 months (108); 46 weeks (109) • *Increase in BMD as well as reduction of markers of bone turnover in longitudinal studies in AS and SpAs (110, 111, 112)* • *Treatment duration: 6 months (infliximab) (110)* *24 weeks (etanercept) (111)* *12 months (infliximab or etanercept) (112)*
Other biologic agents (Adalimumab, Rituximab, Anakinra)	• No clinical studies available

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