Methotrexate and bone mass

M. Mazzantini, O. Di Munno

Sezione di Reumatologia, Dipartimento di Medicina Interna, Università di Pisa, via Roma 67, 56126 Pisa, Italy.

Maurizio Mazzantini, MD, PhD; Ombretta Di Munno, MD, Professor.

Clin Exp Rheumatol 2000; 18 (Suppl. 21): S87-S92.

Key words: Bone mass, methotrexate, osteoporosis, stress fractures, rheumatoid arthritis, bone turnover.

ABSTRACT

In rheumatoid arthritis (RA), methotrexate (MTX) is probably the most frequently used disease-modifying antirheumatic drug. It is also prescribed for other rheumatic and non-rheumatic diseases, such as juvenile RA, psoriatic arthritis, polymyositis, polymyalgia rheumatica, Horton's arteritis, inflammatory bowel disease, etc. MTX has been reported to have negative effects on bone: the term "MTX osteopathy" was first used to refer to a clinical syndrome characterized by stress fractures of the lower extremities, diffuse bone pain, and osteoporosis in children who had been placed on long-term maintenance therapy with low-dose MTX for acute lymphoblastic leukemia. Sporadic reports of similar cases among patients taking low-dose MTX for rheumatic diseases, primarily RA, have appeared more recently. Furthermore, in vitro studies have suggested that MTX may exert toxic effects on osteoblasts.

These findings have raised concern about the long-term effects of MTX on bone. However, densitometric studies in RA patients have so far failed to detect decreased bone mass in patients on MTX treatment.

Introduction

Methotrexate (MTX), a folic acid analogue, is the chemotherapeutic agent used most frequently in the treatment of childhood lymphoblastic leukemia, both for the maintenance of systemic remission and for the treatment of central nervous system involvement, and has been shown to be effective in other malignancies, such as choriocarcinoma and osteogenic sarcoma. The principal mechanism of its antiproliferative action is the competitive inhibition of dihydrofolate reductase, an enzyme that converts dihydrofolate to tetrahydrofolate. Impedance of this reaction interferes with purine synthesis and DNA biosynthesis. The toxicity of MTX to cells is related to both the dose and the duration of exposure. MTX is taken into cells and undergoes polyglutamation, and in this form remains a potent inhibitor of folic acid (1). MTX has been used for more than a decade for the treatment of rheumatoid arthritis (RA) and its efficacy has been proven in controlled clinical trials (2, 3). Many rheumatologists choose MTX as a therapy for RA for its predictable benefit and long-term tolerability: among the disease-modifying antirheumatic drugs (DMARDs) MTX exhibits the most favorable efficacy-to-side effect ratio (4). However, the mechanism of action of MTX in RA and other inflammatory conditions (psoriatic arthritis, polymyositis, juvenile RA, polymyalgia rheumatica) is still not completely understood and only partially imputable to an antiproliferative effect.

Low-dose MTX has only moderate immunomodulatory effects (5). On the other hand, MTX inhibits adjuvant-induced arthritis in animal models and suppresses the passive transfer of adjuvant arthritis by spleen cells (6, 7). MTX inhibits neutrophil chemotaxis in RA patients (8) and is able to attenuate the adhesive interactions between leukocytes and endothelial cells in post-capillary venules during acute inflammation (9), a phenomenon linked to the capacity of MTX to induce adenosine accumulation in fibroblasts by interfering with the aminoimidazole carboxamide ribonucleotide enzyme system, which plays a key role in purine metabolism (10). In fact, adenosine strongly inhibits neutrophil adherence to endothelial cells (11). Furthermore, it has been shown that adenosine inhibits tumor necrosis factor α (TNFα) expression in a monocytic cell line (12), and that monocytes release adenosine after treatment with MTX (13). It is known that interleukin-1 (IL-1) and TNFα play an essential role in the pathophysiology of acute inflammation in RA, and are mediators of cartilage and bone destruction. They also enhance osteoclastic bone resorption. It has been reported that MTX treatment in RA decreases blood mononuclear cell production of the inflammatory cytokines IL-1β and IL-8 and, in parallel, stimulates the release of natural cytokine inhibitors such as IL-1 receptor antagonist (IL-1ra) and soluble TNF receptors (sTNFr) (14). *In vitro* studies find MTX markedly stimulated the differentiation of the human monoblastic leukemia cell line U937, which was associated with enhanced IL-1ra and sTNFr release (15). This finding could explain the clinical anti-inflammatory effects of MTX in RA.

"MTX osteopathy" in malignancies
A syndrome characterized by bone pain and fractures mainly at the lower extremities with osteoporosis and radiological signs resembling scurvy has been named "MTX osteopathy", although its causal relationship with MTX is still debated.
Adverse skeletal manifestations associated with MTX were described initially in some children who received low-dose, long-term oral maintenance therapy for the treatment of acute leukemia at doses of 5-30 mg/m^2 daily or bi-weekly over a 6 month to 3 year period. Ragab *et al.* in 1970 (16) described 5 children out of 11 who developed severe osteoporosis and fractures of the lower extremities. Fractures were multiple in 3 of the 5 patients, and included the distal fibula and the bones of the foot. Severe diffuse bone pain was present in 4 patients. Besides fractures, radiographic features included multiple growth arrest lines and changes simulating scurvy, such as a ring epiphysis, corner sign, and the "white line of scurvy". Following the withdrawal of MTX, symptoms subsided within 3 to 4 weeks and radiographs returned to normal within four months in 4 of the children.
O'Regan *et al.* in 1973 (17) described 5 more children with identical radiographic features, and osteoporosis again was limited to the lower extremities. Stanisavlje *et al.* in 1977 (18) studied 37 children, 20 of whom had osteopenia and bone pain in the lower extremities. Seven patients sustained multiple fractures and 3 had delayed union or even non-union of the distal radius. Two of the fractures healed only after the withdrawal of MTX. Schwartz *et al.* in 1984 (19) described two children, in remission from acute leukemia and Burkitt's lymphoma, who had femoral and tibial fractures.
The prevalence of toxic effects to the skeleton in children receiving MTX for leukemia is not known, but the largest series identified 26 out of 219 children with combinations of bone pain, osteopenia and fractures (20). Fifteen children had fractures: 3 in the upper extremities, 10 in the lower extremities, and 2 in the spine. The osteoporosis was generalized in 2 and confined to the lower extremities in 2. When reviewing associated organ disease, it was noted that these patients had a high incidence of previous multi-system toxicity related to MTX therapy. Eleven had evidence of mild or severe liver disease, 21 had one or more episodes of idiopathic pneumonia, and 2 had renal lithiasis. In several of these cases abnormalities in calcium metabolism were documented, as well as the increased excretion of urinary calcium. Ecklund *et al.* in 1997 (21) investigated retrospectively the occurrence of MTX osteopathy in a study population of 87 patients (mean age 12 years) who were treated with high dose (> 5g/m^2 per cycle), short-term intravenous MTX for osteosarcoma. No patient received steroids. Eight patients exhibited skeletal findings similar to those described in children with leukemia who received low-dose, long-term maintenance MTX. X-rays showed severe osteopenia, dense zones of provisional calcification, multiple bone involvement, and insufficiency fractures (mostly metaphyseal) at the distal tibia, distal radius, proxymal humerus, calcaneus and pubic ramus. Discontinuation of MTX resulted in a radiographically demonstrated improvement.
The pathogenesis of MTX osteopathy is unclear, but it could be related to the antiproliferative effect of MTX. Depression of osteoblast activity leading to disorganization at the site of endochondral bone growth, as in scurvy, has been proposed as a mechanism. Children on long-term MTX have been reported to have normal growth (22). A densitometric study on 95 survivors of childhood acute lymphoblastic leukemia with a median duration of remission of 11 years showed that the whole-body bone mineral content (BMC) and areal bone mineral density (BMD), assessed by dual-energy x-ray absorptiometry (DEXA), were both significantly reduced with respect to controls (23). A subgroup of 33 patients aged 19 years or older at follow-up had the lowest BMC and areal BMD values; in these patients the mean height for age, bone area for height and BMC for bone area were all significantly reduced, which indicated that they had both reduced bone size and a reduced size-adjusted bone mass. With the exclusion of cranial irradiation and bone size, no significant relationships were found between bone parameters and potential causal factors of reduced bone mass, including MTX. Most likely the reductions in bone mineral content and density are related to the severe disease that occurred at the time of bone growth

"MTX osteopathy" in rheumatic diseases
Preston *et al.* in 1993 (24) described two patients, one with psoriasis and the other with RA, who received long-term, low-dose MTX (25 mg/wk and 10 mg/wk, respectively) and who developed features consistent with MTX osteopathy: bone pain, radiological findings of osteoporosis, and stress fractures localized to the tibiae distally. Iliac crest bone histomorphometry showed in both patients reduced osteoid surfaces and osteoid thickness and low bone formation rates, which was in accordance with the results of a study in which the short-term administration of MTX in rats caused a 60% reduction in bone formation rates, the effect on osteoblasts taking the form of reduced osteoid volume and thickness (25). In both patients, when MTX was stopped the symptoms resolved, and rechallenge with MTX in the RA patient resulted in the recurrence of the clinical syndrome.
Eleven additional cases of patients with stress fractures associated with MTX use can be found in the literature: 7 with RA, 1 with psoriatic arthritis, 2 with psoriasis, and 1 with scleroderma (26-33). The role of MTX as a causal agent has been postulated given the similarity of its effects to the clinical syndrome of MTX osteopathy described in children: osteopenia, preferential loss of cortical bone, and stress fractures at the lower limbs typically presenting as sudden, severe pain aggravated by weightbearing. However, since the great majority of the pa-

tients who sustained stress fractures had other well-recognized risk factors for fractures, attributing a causal role to MTX is difficult.

For example, the RA patient described by Zonneveld *et al*. (32) had previous lumbar osteoporotic fractures, low vitamin D_3 plasma levels, and a low functional status. The history of one of the two RA patients described by Meanaut *et al.* (31) included glucocorticoid treatment and a left malleolar, low-trauma fracture, while the history of the other included surgery for a left knee joint deformity and rupture of a right Baker cyst just before the occurrence of the tibial stress fracture. The patient presented by Bologna *et al*. (30) had very low vitamin D_3 levels and was on glucocorticoids. Semba *et al*. (27), Straaton *et al*. (28) and Shapira *et al.* (29) reported stress fractures in patients who were on glucocorticoid treatment, had lower limb deformities due to RA, and who had undergone knee arthroplasty/arthrodesis prior to the stress fractures.

Orthopedic deformities (such as valgisation of the knee and planovalgus of the foot) and reconstructive surgery can predispose to stress fractures, mainly in the tibia and fibula (34-36); this was recognized even before the introduction of MTX therapy. Alonso-Bartolomé *et al*. (37) described 13 RA patients with insufficiency fractures of the tibia and fibula. Eight of them had received MTX therapy. However, 8 of the 13 patients had previous local deformities of the ipsilateral lower limb, generally close to the knee or ankle (valgus subtalar joints, stiff ankles, or valgus deformities of the knee), and one patient also had previous recent surgery in the affected ankle. Furthermore, almost 60% had previous osteoporotic fractures at other sites, and 70% had been on long-term steroid treatment. Thus, in these patients MTX is likely to have played a minor role, if any. It is also to be noted that most of the patients described experienced a complete recovery without withdrawal of MTX therapy.

On the other hand, low-dose MTX has been reported to inhibit fracture healing in an animal model (38), and to have resulted in bone non-union in 2 male RA patients after metatarsal and tibial osteotomy (39). In this latter report prompt healing of the bone occurred after MTX was stopped. However, the hypothesis that MTX may prevent fracture healing by inhibiting osteoblast proliferation and function is not supported by the finding that high-dose MTX, at serum concentrations similar to those used clinically for the treatment of human osteosarcomas, did not show any negative effect on distraction osteogenesis in a rabbit model (40).

There is evidence that even low-dose MTX reaches high concentrations in the bone and synovial membrane in RA patients. Bologna *et al.* (41) examined synovial samples and bone fragments collected during surgery approximately 20 hours after an intra-muscular dose of 10 mg in RA patients, and found that the synovial concentration of MTX was 10.5-fold higher than the simultaneous plasma concentration; and that cortical and trabecular bone concentrations also were 13-fold and 11.5-fold higher, respectively.

MTX, given to rats on a weekly basis for 16 weeks at a dose equivalent to a standard dose for RA in humans, resulted in a significant reduction of bone mass by decreasing bone formation (assessed by serum alkaline phosphatase and osteocalcin levels and histomorphometry) and increasing bone resorption (assessed by urinary hydroxyproline levels and histomorphometry) (42). On mouse bone cells in culture exposed continuously to different concentrations of MTX, diminished osteoblastic cell function occurred (assessed by matrix calcification and supernatant osteocalcin levels) in a dose-dependent manner, without any effect on cell proliferation (43).

In contrast, on human osteoblasts in culture exposed continuously to MTX a strong dose-dependent inhibition of cell proliferation was observed, with no effect on markers of osteoblasts function such as alkaline phosphatase activity or osteocalcin production (44, 45). However, the effect *in vivo* may not reflect these *in vitro* results; in clinical practice MTX administration is not continuous, instead taking the form of sharp, weekly peaks. A dose-dependent toxic effect of MTX on UMR 106 rat osteosarcoma cells was observed after exposure to the concentrations found in patients with RA but, once again, the experiment involved continuous exposure (46). No significant inhibition of alkaline phosphatase activity was seen at any MTX concentration. Interestingly, the effect of MTX on cell viability was prevented by the addition of folinic acid at the time of the addition of MTX and then 4 hours later, which suggests that the mechanism of MTX toxicity in UMR 106 cells involves the inhibition of folic acid metabolism; this may be the case for human osteoblasts as well.

In rats with adjuvant-induced arthritis the daily administration of MTX alleviated inflammatory edema without hampering normal bone growth as measured by the lumbar and femoral BMD values. While serum osteocalcin levels were decreased and urinary deoxypyridinoline levels were increased in the arthritic control rats, in those treated with MTX these bone markers remained at the levels of normal rats. Decreases in the mineral apposition and bone formation rates and increases in the trabecular osteoclast number and surface were prevented by MTX (47).

In summary, MTX seems to maintain bone mass by preventing a decrease in bone formation and an increase in bone resorption in the adjuvant arthritic rats: the effects of MTX on bone turnover in arthritic rats are suggested to differ greatly from those in normal rats. This has been confirmed by others (48). Low-dose weekly MTX therapy in rats with adjuvant-induced arthritis restored the decreased osteogenic activity of bone marrow cells and reduced their increased bone resorptive activity. These changes resulted in a significant increase in femoral BMD.

Similar results have been observed for the markers of bone metabolism in humans. Thirty female patients with active RA were given MTX at a weekly dose of 10-15 mg. Deoxypyridinoline and bone alkaline phosphatase levels were assessed at baseline and after 3 and 9 months of MTX treatment: at 9 months deoxypyridinoline was significantly reduced and bone alkaline phosphatase was increased compared with pre-MTX levels. These changes were accompanied by a significant improvement in RA activity

(49). Thus, MTX in RA patients may have a protective effect on bone by controlling disease activity.

Densitometric studies in RA patients

Katz *et al.* in 1989 (50) measured BMD using dual photon absorptiometry in 10 female patients with RA who had received a mean cumulative MTX dose of 625 mg, and in 19 matched controls. No significant differences were detected between the groups in lumbar spine, femoral neck, inter-trochanteric or Ward's triangle BMD values.

Buckley *et al*. in 1997 (51) reported on BMD changes (measured by DEXA) in both male and female RA patients treated with MTX (n = 68) (mean cumulative dose 1375 mg) or with another DMARD (n = 27) after a follow-up of 3 years. The change in BMD, adjusted for age, sex, Health Assessment Questionnaire results and prednisone use, was similar in MTX and non-MTX treated patients, with a difference of -2.0% (p = 0.359) in the lumbar spine and of +0.85% (p = 0.58) in the femoral neck. However, patients treated with prednisone ≥ 5 mg/day plus MTX showed an 8.08% greater loss of BMD in the lumbar spine than patients treated with a similar dose of prednisone without MTX (p=0.004), which suggests that MTX may increase trabecular bone loss in glucocorticoid-treated patients by augmenting their inhibitory effect on osteoblast function. This study has some limitations, however: estrogen replacement therapy was used concurrently in about 25% of the patients, and calcium and vitamin D supplementation in about 50%. Furthermore, information about the timing of glucocorticoid and MTX administration and disease activity during follow-up was lacking. Nonetheless, the results seem to exclude any effect of MTX at the femoral site.

With regard to the suggested additive effect of MTX and glucocorticoids on vertebral BMD, it is to be noted that in 12 polymyalgia rheumatica patients treated with prednisone (mean cumulative dose after 1 year 1.84 g) and MTX 10 mg weekly, vertebral BMD, as assessed by DEXA, did not change after 1 year versus baseline values: 0.75 g/cm^2 versus 0.76 g/cm^2 (p = NS) (52). On the contrary, significant bone loss occurred in 12 patients treated with prednisone alone (mean cumulative dose 3.2 g): 0.78 g/cm^2 versus 0.82 g/cm^2 (p = 0.002).

Carbone *et al*. in 1999 (53) compared lumbar spine and femoral neck BMD, assessed by DEXA, in 2 groups of postmenopausal RA patients, one being treated with MTX (n = 10; treatment duration > 3 years) and one which was not (n = 10). No significant differences were detected, but the results were not corrected for age, which was significantly higher in those not receiving MTX.

Mazzantini *et al.* (54) recently reported the results of a 2-year, longitudinal study aimed at evaluating lumbar BMD changes assessed by DEXA in female RA patients who had recently started DMARD therapy. Exclusion criteria included any disease or drug that could affect bone turnover. Glucocorticoids were allowed if started more than 12 months prior to the study entry, at a dose not exceeding 7.5 mg prednisone or the equivalent. The characteristics and baseline clinical data for the two treatment groups are given in Table I. After two years, 22 patients treated with MTX (mean cumulative dose 1209 mg) and 18 patients treated with another DMARD had lost a comparable amount of bone, the difference being -0.9% (p = NS) (Fig. 1). No correlation was found between the cumulative dose of MTX and the changes in BMD after 2 years (r = -0.14, p = NS). This study sought to minimise the presence of other known factors that could interfere with bone metabolism. In particular, patients taking glucocorticoids at baseline had to continue their treatment during the entire study period at a dose range equivalent to 5.0 - 7.5 mg prednisone, and those starting or stopping glucocorticoids were excluded from the densitometric analysis as significant changes in BMD could have occurred.

Conclusions

Although MTX has shown significant effects in animal models and in osteoblast and osteoblast-like cell cultures, present data does not support the hypothesis that low-dose MTX negatively affects bone mass in RA. This is probably due to the capacity of MTX to suppress factors that in RA are involved in both inflammation and bone resorption, as discussed in the introduction to this article. Some questions, however, still need to be answered before we can definitively assume that MTX is safe for the skeleton. First, what is the effect of MTX on bone in patients with inactive RA, where no compensatory action of MTX on disease activity is taking place ? Second, what is the effect of MTX in diseases not affecting bone mass *per se*,

Table I. Characteristics and baseline clinical data for 2 groups of RA patients, one treated with methotrexate (MTX) and one with other DMARDs (ref. 54).

	MTX (n = 22)	Other DMARDs (n = 18)	P
Age (years)	59 ± 9	57 ± 12	NS
BMI	23.2 ± 1.8	23.8 ± 1.8	NS
N° in menopause	17	14	NS
Years of menopause	13 ± 7	11 ± 8	NS
Disease duration	9 ± 9	9 ± 10	NS
N° taking GC	18	15	NS
Years of GC	3.3 ± 1.6	3.0 ± 2.2	NS
RF+	16	15	NS
Ritchie index	11 ± 4	11 ± 4	NS
N° swollen joints	10 ± 5	12 ± 4	NS
ESR (mm/h)	44 ± 19	41 ± 20	NS
CRP (mg/dl)	2.7 ± 2.1	2.6 ± 2.3	NS
N° with active disease	18	14	NS
Lumbar BMD (g/cm^2)	0.98 ± 0.13	1.05 ± 0.16	NS
T-score	-1.7 ± 1.1	-1.5 ± 1.5	NS

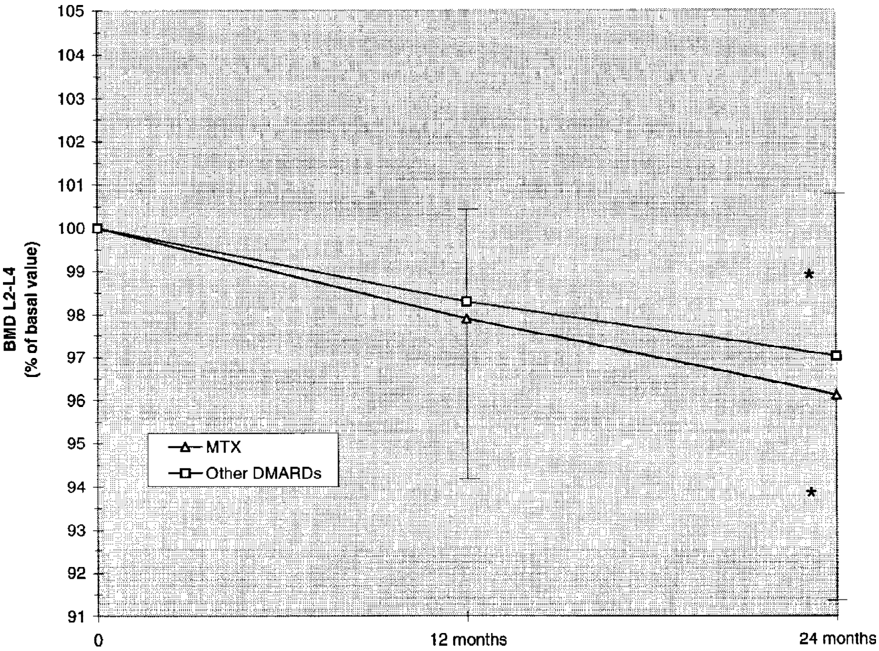

Fig. 1. Changes in lumbar BMD, expressed as % of the basal value, after 12 and 24 months, in the two treatment groups of RA patients (* p < 0.001 vs basal value) (ref. 54).

such as polymyositis and polymyalgia rheumatica ? Third, could folinic acid supplements help to prevent bone loss in MTX-treated patients, since it has been shown to prevent MTX-induced toxicity in osteoblats-like cells *in vitro* (46)?

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