Effect of cyclosporine A on bone density in female rheumatoid arthritis patients: results from a multicenter, cross-sectional study

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Objective

To analyze the influence of cyclosporine A (CYA) on bone using data from a large multicenter, cross-sectional study on bone mineral density (BMD) in rheumatoid arthritis (RA)

Methods

We selected 558 female patients with RA and divided them into two groups on the basis of CYA use: those who had never used CYA (n = 467) and CYA users (n = 91; users for < 24 months n = 50; users for > 24 months n = 41). Demographic, disease and treatment-related variables were collected for each patient. BMD was measured at the lumbar spine and proximal femur using dual x-ray absorptiometry. Data was analyzed by means of a univariate and multivariate statistical procedure. Osteoporosis (OP) was defined as BMD < -2.5 T score.

Results

The frequency of OP among non-CYA users and CYA users was 28.2% and 33.3% (p=NS) for the lumbar spine, and 34.2% and 31.3% (p=NS) for the femoral neck, respectively. The prevalence of fragility fractures was not significantly different between the two groups. Mean values for the T-score at either the lumbar spine or the femoral neck were comparable in the two groups, even after adjustment for age, menopausal status, body mass index (BMI), Health Assessment Questionnaire (HAQ) score and steroid use. The generalized linear model showed that age, BMI and the HAQ score were significant independent predictors of BMD at the lumbar and femoral levels, whereas CYA use was not. Logistic analysis showed that only age, the HAQ score and BMI were significantly associated with the risk of OP. However, the duration of CYA therapy > 24 months was associated with an adjusted decreased lumbar BMD and a significantly decreased femoral neck BMD (p = 0.01). The frequency of femoral neck OP in patients on CYA for > 24 months was significantly higher than in patients on CYA for < 24 months: 46.4% vs. 19.44% (p=0.03), while the prevalence of fragility fractures did not differ significantly: 23.1% vs. 16.6%, respectively (p=NS). Logistic analysis showed that CYA use was an independent predictor of osteoporosis at the femoral site.

Conclusion

Long-term CYA therapy may have negative effects on BMD in female RA patients.

Key words

Rheumatoid arthritis, osteoporosis, bone loss, cyclosporine A.

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Received on July 12, 2006; accepted in revised form on May 4, 2007 .

Competing interests: none declared.

Introduction

After the discovery of its immunosuppressive actions, which revolutionized transplantation medicine, cyclosporine A (CYA) was also widely investigated for the treatment of various immunological and rheumatic diseases. CYA has proved to be effective in both advanced and early rheumatoid arthritis (RA) and to retard the radiological progression of the disease (1-4). At the doses commonly used for the treatment of RA, the toxicity is considered to be manageable, even though caution must be exercised in the presence of renal impairment and hypertension. Some concern remains regarding the potential bone toxicity of CYA, however. Osteoporosis and fractures are common in allogeneic organ transplantation and CYA may contribute to the pathogenesis of bone fragility (5). However, in most transplant recipients CYA is co-administered with other immunosuppressive drugs such as glucocorticoids, which are known to adversely affect bone, therefore making it difficult to address the question of the skeletal effects of CYA in clinical studies. *In vivo* animal experiments demonstrate a high turnover disease with loss of bone volume (6-8). In transplant recipients, some studies have indicated a deleterious effect of CYA on bone mass (9-11), whereas other clinical trials have suggested that monotherapy with CYA may not be associated with bone loss (12-16). Moreover, a study in kidney transplant patients reported that CYA may actually counterbalance the adverse effects of GCs on the skeleton (17). Thus, the risk of osteoporosis in transplantation as a result of treatment with CYA remains controversial.

With the exception of the study carried out by Ferraccioli *et al*. (18) on patients with early and aggressive erosive disease and a poor previous response to treatment with MTX, there are no studies specifically addressing the effect of CYA on bone metabolism in RA. Ferraccioli reported an average decline in bone mineral density (BMD) of 4% in 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 the BMD increased by 3.9%.

The aim of the present study was to analyze the influence of CYA on bone based on data from a large multicenter, cross-sectional study on BMD in RA (19).

Materials and methods

Subjects

The data analyzed in the present study were collected by 21 Italian rheumatology centers during a large cross-sectional survey on bone mass in 925 RA patients (19). The only inclusion criteria were female sex and an established diagnosis of RA in accordance with the 1987 revised criteria of the American College of Rheumatology (ACR). Women who had undergone a hysterectomy were excluded. Other exclusion criteria were diabetes, severe hepatic or renal disease, and diseases of the thyroid gland. Patients who were unable to walk without assistance and those who had undergone total bilateral hip replacement were also excluded. In order to evaluate the effect of CYA on bone mass in comparison to other drugs not affecting bone, we excluded from analysis 246 patients treated with methotrexate, even though a negative effect of this drug on bone seems unlikely (20, 21).

Predictive variables

At recruitment, data were collected on age, body weight, height, and age at surgical or natural menopause. Disease-related variables included disease duration, oligo- versus polyarticular involvement, involvement of weight-bearing joints, presence of subchondral erosions (hands or forefeet), presence of extraarticular manifestation, number of swollen joints (where metacarpophalangeal, metatarsophalangeal, and interphalangeal were considered as 3 single joints), and a history of major (total hip replacements) or minor orthopedic surgery related to RA. The functional evaluation included staging of the disease according to Steinbrocker's classification (22). The measure of self-reported functional status was based on the Health Assessment Questionnaire (HAQ).

For each postmenopausal patient, any

previous history of fragility fractures (femur, wrist, ribs, pelvis, humerus) was obtained. In all cases, a lateral radiograph of the dorsal and lumbar spine was taken to check for previous vertebral fractures. A vertebral fracture was defined as a reduction of at least 25% in vertebral height.

Steroid usage was carefully evaluated. Patients were subdivided into users and non-users, and for each user the current and cumulative dose and the treatment duration were recorded.

Finally, patients were interviewed about any past or current use of drugs that might affect bone metabolism including estrogens, bisphosphonates, calcium, vitamin D, calcitonin, anabolic steroid, fluoride, and thiazides. To examine the effect of CYA on bone, patients were divided into two groups: those who had never been treated with CYA, and current CYA users. Current CYA users were further categorized into two subgroups depending on the duration of CYA therapy: less than 24 months or more than 24 months.

Bone mass measurements

BMD at the lumbar spine and proximal femur were measured at the time of recruitment using Hologic, Lunar or Norland scanners and applying the dual x-ray absorptiometry (DXA) technique. T-scores were calculated by each center after comparison with reference values supplied by the manufacturer. Osteoporosis was defined as a T-score > -2.5 standard deviations, according to the definition of the World Health Organization (23).

Statistical analysis

The baseline characteristics were compared in the two groups (non-CYA users and current CYA users) using Student's *t*-test for unpaired data in the case of continuous variables and the chi-squared test for categorical variables. Significance was reported at $p \leq 0.05$. Adjusted means were also calculated in the different groups to obviate the effects of the main confounding variables using the analysis of covariance. A generalized linear model was applied to analyze the predictors of BMD in RA. The relative risks associated with osteoporosis were assessed by logistic models in which the presence of osteoporosis in at least one region of interest was the dependent variable. The inclusion criteria for variables in the multivariate analyses were: (i) statistical significance assessed by univariate analysis, and (ii) the clinical relevance of the given variable on the outcome variable. All analyses were performed usingh SAS software.

Table I. Demographic and clinical data (mean ± SD or %) for the rheumatoid arthritis patients grouped by CYA use.

	Non-CYA users (n = 467)	CYA users (n = 91)	*p*
Age (yrs.)	58.8 ± 12.5	52.7 ± 13.5	< 0.0001
Body mass index (Kg/m^2)	24.8 ± 4.1	25.1 ± 4.0	NS
Post-menopausal (%)	76.5	61.5	0.003
Menopause duration (yrs.)	14.7 ± 9.1	13.2 ± 8.7	NS
RA duration (yrs.)	9.0 ± 8.0	6.0 ± 5.6	0.02
Rheumatoid factor + (%)	66.3	75.8	0.02
ESR (mm)	30 ± 21	40 ± 20	< 0.0001
CRP (mg/dl)	3.3 ± 5.4	4.4 ± 10.6	NS
HAQ score	1.2 ± 0.9	1.2 ± 0.8	NS
N° of swollen joints	3.1 ± 3.6	4.5 ± 4.6	0.006
Presence of erosion (%)	65.5	76.7	0.03
Steinbrocker functional class (% in class III or IV)	26.9	26.4	NS
Steroid use (%)	58.7	68.1	NS
Duration of steroid use (yrs.)	3.8 ± 4.0	2.5 ± 2.0	0.005
Mean daily steroid dose (mg)	5.2 ± 4.4	5.4 ± 4.5	NS
Cumulative steroid dose (g)	8.4 ± 11.2	6.5 ± 9.8	NS

Results

Univariate analysis

The study cohort was comprised of 558 female RA patients. On the basis of CYA use, two groups were identified: non-CYA users (n = 467) and CYA users (n = 91). Among CYA users, 50 patients had been treated for less than 24 months, and 41 for more than 24 months.

Table I shows the demographic and clinical data (mean ± SD or %) for the patients, which differed significantly between the two groups. CYA users were significantly younger (52.7 ± 13.5 *vs.* 58.8 ± 12.5 years, $p < 0.0001$) and fewer were postmenopausal (61.5% *vs.* 76.5%, $p = 0.003$) than the non-CYA users. Furthermore CYA users had a significantly shorter disease duration (6.0 ± 5.6 *vs.* 9.0 ± 8.0 years, $p = 0.02$) and duration of steroid use (2.5 ± 2.0 *vs.* 3.8 ± 4.0 years, $p = 0.005$); they also exhibited a higher ESR (40 ± 20 mm vs 30 ± 21, $p < 0.0001$), a higher number of swollen joints (4.5 ± 4.6 *vs.* 3.1 ± 3.6, $p = 0.006$) and a higher proportion of erosive disease (76.7% *vs.* 65.5%, $p = 0.03$).

True clinical remission according to the ACR criteria (24) was diagnosed in 9/91 CYA-treated patients (9.9%) and in 79/467 non-CYA treated patients (15.9%); the difference was not statistically significant ($p = 0.14$).

No difference was detected between the two groups with regard to oligo- versus polyarticular involvement, involvement of weight-bearing joints, the presence of extra-articular manifestations, and a history of major or minor orthopedic surgery related to RA.

At the time of recruitment, 50.2% of postmenopausal patients (207 out of 413) were on treatment with drugs affecting bone metabolism: calcium, 9.3% (n = 52); vitamin D, 17.2% (n = 95); bisphosphonates, 12.4% (n = 69); and hormone replacement therapy, 1.8% (n = 10); 19 women were being treated with drugs in different combinations. No statistically significant difference was observed between the two groups in this regard.

In postmenopausal women, we detected a total of 108 fragility fractures in

68 patients (16.5%). Fractures were detected in 57 (15.9%) postmenopausal patients who had never used CYA and in 11 (19.6%) who had used CYA (p = NS).

The mean unadjusted values for the T-score (± SD) at either the lumbar spine or the femoral neck were comparable in the two groups of patients (Table II): the lumbar spine T-score was 1.65 ± 1.40 among non-CYA users and -1.67 ± 1.56 among CYA users (p = NS); the femoral neck T-score was -1.97 ± 1.17 and -1.95 ± 1.21, respectively (p = NS). The mean T-scores (± SE) at the two sites, when adjusted for age, menopausal status, BMI, HAQ score and steroid use, were also comparable between the two groups: the lumbar spine T-score was -1.59 ± 0.09 among non-CYA users and -1.69 ± 0.17 among CYA users (p = NS); the femoral neck T-score was -1.95 ± 0.07 and -2.08 ± 0.09, respectively (p = NS). The frequency of osteoporosis among non-CYA users and CYA users was 28.2% and 33.3% (p = NS) for the lumbar spine, and 34.2% and 31.3% (p = NS) for the femoral neck, respectively (Table II).

The CYA users, when subdivided based on treatment duration, were comparable for age and other demographic and clinical characteristics (Table III). Fragility fractures were detected in 5 (16.6%) postmenopausal patients on CYA for < 24 months and in 6 (23.1%) patients on CYA for > 24 months (p = NS). BMD was different at both the lumbar spine (-1.42 ± 1.62 *vs*. -2.12 ± 1.37, p = 0.08) and femoral neck (-1.62 ± 1.16 *vs*. -2.37 ± 1.17; p = 0.01) in patients on CYA for < 24 months (n = 50) compared to those on CYA for > 24 months (n = 41); at the femoral neck the difference reached statistical significance. The significance level of these differences remained unchanged after adjustment for age, menopausal status, BMI, the HAQ score and steroid use. The frequency of osteoporosis among patients who were on CYA for < 24 months compared to those on CYA for > 24 months was 30.9% and 37.5% (p = NS) for the lumbar spine and 19.4% and 46.4% (p = 0.03) for the femoral neck, respectively (Table IV). The results remained unchanged after the exclusion of patients on bisphosphonates and those on hormone replacement therapy.

Table II. BMD (T-score, expressed as means ± SD) in RA patients grouped according to CYA use (never used *vs*. treated with), and the prevalence (%) of osteoporosis and osteopenia in these patients.

	Non-CYA users (n = 467)	CYA users (n = 91)	p
Lumbar spine	-1.65 ± 1.40	-1.67 ± 1.56	NS
Femoral neck	-1.97 ± 1.17	-1.95 ± 1.21	NS
Lumbar spine, %			
Normal	29.1	31.8	
Osteopenic	42.7	34.9	
Osteoporotic	28.2	33.3	NS
Femoral neck, %			
Normal	19.4	15.6	
Osteopenic	46.4	53.1	
Osteoporotic	34.2	31.3	NS

Table III. Demographic and clinical data (mean ± SD or %) for the two groups of rheumatoid arthritis patients treated with CYA for < 24 months or > 24 months.

	CYA < 24 months (n = 50)	CYA > 24 months (n = 41)	p
Age (yrs.)	51.6 ± 14.4	53.9 ± 12.3	NS
Body mass index (Kg/m^2)	24.5 ± 4.0	25.8 ± 3.9	NS
Post-menopausal (%)	60.0	63.4	NS
Menopause duration (yrs.)	12.4 ± 8.3	14.2 ± 9.1	NS
RA duration (yrs.)	6.0 ± 5.8	7.0 ± 6.0	NS
Rheumatoid factor + (%)	74.0	78.1	NS
ESR (mm)	38 ± 18	43 ± 20	NS
CRP (mg/dl)	5.2 ± 11.5	3.5 ± 9.3	NS
HAQ score	1.2 ± 0.8	1.3 ± 0.7	NS
N° of swollen joints	4.5 ± 5.0	4.5 ± 4.0	NS
Presence of erosion (%)	72.0	82.5	NS
Steinbrocker functional class (% in class III or IV)	27.0	26.2	NS
Steroid use (%)	70.0	65.6	NS
Duration of steroid use (yrs.)	2.0 ± 20	2.6 ± 2.0	NS
Mean daily steroid dose (mg)	5.4 ± 4.4	5.4 ± 4.5	NS
Cumulative steroid dose (g)	6.8 ± 12.2	6.1 ± 5.5	NS

Multivariate analysis

To analyze the independent effect of different covariates that could influence the course of/impact on osteoporosis in RA, we performed a multivariate analysis of the data. The generalized linear model at the lumbar and femoral sites was applied to identify independent predictors of BMD in our population. Due to missing values, 430 observations were evaluated for the lumbar spine and 387 for the femoral neck. Age, BMI and HAQ score were significant independent predictors of BMD at the lumbar spine and femoral sites in this model, whereas CYA use was not, even after the exclusion of patients on bisphosphonates or hormone replacement therapy (Table V). The generalized linear model was also applied in the 91 patients taking CYA: age, BMI and HAQ were significant independent predictors of osteoporosis at the lumbar spine and femoral levels, while CYA use was an independent predictor of osteoporosis at the femoral level (Table VI).

The logistic model was also applied using the presence of osteoporosis in at least one site (*i.e*., the lumbar spine or femoral neck) as the dependent variable. In this model (478 observations), age (OR 1.06, 95% CI: 1.04 -1.07), the HAQ score (OR 1.50, 95% CI: 1.20-

Table IV. BMD (T-score, expressed as means ± SD) in RA patients grouped according to CYA use (< 24 months or > 24 months), and the prevalence (%) of osteoporosis and osteopenia in these patients.

	CYA < 24 months (n = 51)	CYA > 24 months (n = 40)	*p*
Lumbar spine	-1.42 ± 1.62	-2.12 ± 1.37	0.08
Femoral neck	-1.62 ± 1.16	-2.37 ± 1.17	0.01
Lumbar spine, %			
Normal	35.7	25.0	
Osteopenic	33.3	37.5	
Osteoporotic	31.0	37.5	NS
Femoral neck, %			
Normal	19.5	10.7	
Osteopenic	61.1	42.9	
Osteoporotic	19.4	46.4	0.03

Table V. Generalized linear model of lumbar and femoral BMD in 546 women with rheumatoid arthritis.

	Lumbar spine		Femoral neck	
Variable	β coefficient	*p*	β coefficient	*p*
Age	-0.02	0.0001	-0.03	0.0001
Menopause	0.16	NS	0.14	NS
BMI	0.09	0.0001	0.09	0.0001
HAQ	-0.30	0.004	-0.18	0.04
Disease duration	0.0009	NS	-0.0004	NS
Steinbrocker stage	-0.001	NS	0.17	NS
Use of steroids	0.20	NS	0.11	NS
Use of CYA*	0.10	NS	0.14	NS

*When patients on bisphosphonates or hormone replacement therapy were excluded, the β coefficient was 0.08 (*p* = NS) for lumbar spine BMD and –0.02 (*p* = NS) for femoral neck BMD.

Table VI. Generalized linear model of lumbar and femoral BMD in 91 women with rheumatoid arthritis who were taking CYA.

	Lumbar spine		Femoral neck	
Variable	β coefficient	*p*	β coefficient	*p*
Age	-0.02	0.0001	-0.03	0.0001
Menopause	0.16	NS	0.14	NS
BMI	0.09	0.0001	0.09	0.0001
HAQ	-0.29	0.004	-0.18	0.04
Disease duration	-0.0001	NS	0.0004	NS
Steinbrocker's stage	-0.005	NS	0.07	NS
Use of steroids	0.20	NS	0.16	NS
Use of CyA*	0.12	NS	0.21	0.04

*After exclusion of the patients on bisphosphonates or hormone replacement therapy, the β coefficient was 0.12 (*p* = NS) for lumbar spine BMD and 0.22 (*p* = 0.035) for femoral neck BMD.

1.88), and BMI (OR 0.88, 95% CI: 0.84-0.93) were significantly associated with the risk of osteoporosis.

Discussion

To the best of our knowledge, the present study is the first to assess the effect of CYA on bone in RA patients. CYA has been assumed to exert a negative effect on the bone, mainly by accelerating bone resorption. If this is true, then careful attention must be paid when treating RA patients, who are known to be at risk of developing osteoporosis and fractures by the action of the disease itself. The effect of CYA on bone could be clinically relevant in this setting. Comparing patients who were treated with CYA to those who were not, we found that the mean adjusted values for the T-score at the lumbar spine and femoral neck were comparable in the two groups of RA patients, and that the proportion of patients with osteoporosis did not differ. However, when examining BMD and the prevalence of osteoporosis in patients treated with CYA for a longer or shorter period, the results suggest that CYA may cause bone loss when given for more than two years. In fact, patients given CYA for more than 2 years had a lower adjusted lumbar and femoral ($p < 0.01$) BMD, and a significantly ($p < 0.03$) higher percentage of them had osteoporosis compared to those given CYA for a shorter period of time. On the basis of this data, we conclude that CYA has a negative effect on bone in RA patients; further long-term longitudinal studies should be undertaken to confirm or negate this conclusion.

The present study has some limitations; since it was not a longitudinal study it may not have accurately assessed the role of other factors causing bone loss in RA, such as disease activity and functional disability. A second drawback is that when they were enrolled, the duration of current or past DMARD therapy was assessed only semi-quantitatively (*i.e.* < 24 months or > 24 months) in the patients, and mean and cumulative doses of each DMARD were not recorded. As a consequence, we do not have information regarding the exact duration of CYA treatment and the mean doses of CYA in our patients. Based on accepted clinical practice, it is likely that most of our patients were taking CYA at a dose of 2-4 mg/Kg/day, and therefore our results should not be extrapolated to patients being treated with CYA at other dosages.

On the other hand, at least two aspects of this study lend strength to its results. The first is the large number of patients (n = 558) from various clinics across Italy being studied (19), 91 of whom were being treated with CYA. The second is that the two subgroups of patients who were analyzed on the basis of the length of their CYA treatment were closely comparable in terms of age and other demographic and clinical characteristics (including GC therapy), which limits the possibility that the

differences found in BMD and osteoporosis were due to confounding factors. Moreover, multivariate analysis showed that CYA use was a significant independent predictor of osteoporosis at the femoral level, along with age, BMI and the HAQ score.

The mechanisms by which CYA can induce bone loss are not fully understood, but extensive reviews have been dedicated to this issue in both rheumatic and transplant patients (25-27). It has been suggested that CYA may increase bone resorption by increasing RANKL and decreasing osteoprotegerin (28). Future longitudinal studies should focus on bone turnover by measuring the markers of bone resorption and formation, as was recently done in renal transplant patients (29); serum osteocalcin and urinary N-telopeptides were measured over a one-year period in 115 patients who were receiving either CYA or sirolimus as their primary therapy in combination with azathioprine and glucocorticoids or mycophenolate mofetil and glucocorticoids. Urinary excretion of N-telopeptides and the concentrations of serum osteocalcin were consistently higher in CsA-treated patients, and were significantly different at week 24 for N-telopeptides and at weeks 12, 24, and 52 for osteocalcin. These results provide further evidence that CYA may increase bone turnover in transplant patients, although the overall effect of CYA on bone still remains controversial.

In conclusion, long-sterm CYA therapy may have a negative effect on BMD in female RA patients.

Appendix

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