Short Term Whole Body Retention in Relation to Rate of Bone Resorption and Cartilage Degradation After Intravenous Bisphosphonate (Pamidronate) in Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** Bisphosphonates (BP) inhibit osteoclast-mediated bone resorption, and have been reported to decrease the rate of cartilage degradation. The anti-resorptive effect of BP is determined by the amount of BP retained by the skeleton. In rheumatoid arthritis (RA) the uptake is not confined only to the skeleton, but BP is also retained in joints, which could have implications for dose regimens. We investigated the whole body retention (WBR) of pamidronate and its relationship to bone resorption and cartilage degradation in patients with active RA.

Methods. Twenty-six patients received placebo, 45 mg, or 90 mg intravenous pamidronate. Serum and urine samples were collected before and for 12 days after drug administration. Rate of bone resorption was assessed by the biochemical markers: serum carboxy terminal cross-linked telopeptide of type I collagen, urinary carboxy terminal cross-linked telopeptide of type I collagen normalized to creatinine and urinary amino-terminal telopeptide of type I collagen normalized to creatinine; and rate of cartilage degradation by urinary carboxy terminal telopeptide of type II collagen normalized to creatinine. WBR was derived from urinary excretion of pamidronate data.

Results. Pamidronate induced a rapid and sustained decrease in the level of biochemical markers of bone resorption and cartilage degradation. The mean WBR of pamidronate was 69% of the administered dose, and showed a remarkably wide range (41-96%). The decrease in rate of bone resorption, but also rate of cartilage degradation appeared to be related to the WBR of pamidronate.

Conclusion. This is the first study in which the effect of BP treatment has been studied in relation to the amount of BP retained by the body in patients with active RA. The total amount of BP retained by the body shows a remarkably wide range and is comparable with literature on patients with osteoporosis. The apparent relationships between the amount of BP retained by the body and the effect could have implications for therapeutic regimens in patients with RA. (J Rheumatol 2004;31:1732–7)

Key Indexing Terms:
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Rheumatoid arthritis (RA) is characterized by cartilage degradation in affected joints as well as localized and generalized bone loss. Bisphosphonates (BP) are synthetic compounds that inhibit osteoclast-mediated bone resorption, and are used widely in the management of osteoporosis[1]. BP suppress the arthritic response in animal models of adjuvant arthritis and have been reported to decrease joint symptoms (pain and swelling), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) in patients with RA[2]. However, this has not been consistently observed[3,4].

Recently, Lehmann, *et al* showed a significant effect of the BP alendronate and ibandronate on cartilage turnover assessed with a specific cartilage marker (urinary type I collagen II telopeptide) in postmenopausal women[5], and zoledronate was reported to induce a rapid decrease of this marker in patients with Paget's disease[6]. Therefore, it seems that BP can reduce cartilage degradation. It remains to be

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unraveled, however, whether this potential chondroprotective effect of BP is direct or secondary to inhibition of bone resorption.

In patients with RA, bone metabolism is related to disease activity[7]. In active RA there is an imbalance between bone formation and resorption that may contribute to bone loss[7]. Compared, however, to osteoporosis, it is not known whether this local and generalized bone resorption and the extra involvement of cartilage degradation in RA have consequences for the therapeutic regimens of BP that should be used. Considering the specific pharmacology of BP, the antiresorptive effect is determined by the amount of the BP retained at the skeleton[8]. This amount can be calculated by studying whole body retention (WBR) of BP, which in osteoporosis, is equal to the amount of BP bound specifically to the skeleton. In contrast, in RA there is preferential uptake of BP in affected joints[9]. This uptake may be the result of an increased number of periarticular binding sites resulting from localized bone resorption. WBR of BP in patients with RA should therefore represent the sum of skeletal and joint retention. We investigated the WBR of intravenous (IV) pamidronate and its relationship to bone resorption and cartilage degradation in patients with active RA.

MATERIALS AND METHODS

Patients. This study was part of an investigation into clinical and biochemical effects of IV pamidronate in patients with RA. The design, patient characteristics, and results of the original study have been reported[10]. In brief, 26 patients with RA, according to 1987 American College of Rheumatology criteria[11] were randomly allocated on a double blind basis to receive a single IV infusion of placebo, 45 mg (group 1), or 90 mg (group 2) pamidronate in 250 ml 0.9% NaCl over 3 hours. Patients had moderate or active RA as defined by a disease activity score > 2.4[12]. The study was approved by the ethical committee of the Slotervaart Hospital. All patients gave written informed consent before entering the study. Baseline patient characteristics of the 3 groups were not significantly different[10] (Table 1). Blood and urine (second void) were collected at days –3, 0, 4, 7, and 12 for measurement of biochemical markers, and 24 h urine samples were collected at day 1, 6, and 12, for pamidronate determination.

Bioanalyses. Bone resorption was assessed by the measurement of 3 different biochemical markers. Serum ß-isomerized carboxy terminal telopeptide of type 1 collagen (ß-CTx) was determined by an ECLIA (CrossLaps kit, Roche diagnostics). Urinary N-terminal telopeptide of type 1 collagen (uNTx) was determined by ELISA (Osteomark kit, Ostex International, Seattle, WA, USA). Urinary ß-CTx was measured by ELISA (Osteometer Crosslaps kit, Nordic Bioscience Diagnostics, Herlev, Denmark). Cartilage degradation, measuring carboxy terminal telopeptide of type II collagen breakdown products in urine (CTxII), was assessed by ELISA (Osteometer, Cartilaps kit, Nordic Bioscience Diagnostics, Denmark). Urinary markers were corrected for urinary creatinine excretion (uNTx/Cr, uß-CTx/Cr, and uCTXII/Cr).

Pamidronate was determined in the 24 h urine samples by a validated HPLC/fluorimetry method[13].

WBR. WBR of pamidronate on day 1 (WBR_1) was calculated as IV dose minus the amount excreted into urine during 24 h after start of treatment as described[14]. The amount excreted into urine on the second day ($Ae_{,2}$) and the elimination rate constant for the amount excreted into urine (k) during 12 days were calculated using linear regression analysis on the urinary excretion data from day 6 and day 12. The amount of pamidronate retained by the skeleton over time, WBR_t, was estimated subsequently according to the following equation:

$$WBR_t = WBR_1 - {}_o\Sigma^t (Ae_{,2}) * e^{-k(t-1)}$$

Bone and cartilage resorption. Rate of bone resorption before and after treatment was expressed as percentage of prevalent (baseline) value of sß-CTx, uß-CTx/Cr, and uNTx/Cr and as an absolute value of these measures. Rate of cartilage degradation was expressed as percentage of prevalent (baseline) value of uCTXII/Cr and as an absolute value of this measure.

Statistics. Results are expressed as means ± standard deviation. Apparent differences between group 1 and group 2 were investigated with Student's t-test and Wilcoxon Rank test. All statistics were performed using the SPSS software (version 10.0); (uncorrected) $p < 0.05$ was regarded statistically significant.

Table 1. Baseline patient characteristics. Results are expressed as mean ± standard deviation.

Treatment Group	Placebo	45 mg	90 mg
n	9	8	9
Male/Female	3/6	4/4	1/8
Age, yrs	66 ± 15	58 ± 13	56 ± 15
Weight, kg	79 ± 9	69 ± 19	68 ± 7
Clcr, ml/min	78 ± 17	100 ± 35	94 ± 43
uCTXII/Cr, µmol/mmol	0.48 ± 0.23	0.56 ± 0.38	0.55 ± 0.27
sCTx, µg/ml	0.44 ± 0.18	0.43 ± 0.27	0.45 ± 0.23
uCTx/Cr, µg/mmol	369 ± 110	413 ± 276	417 ± 139
uNTx/Cr, nmol/mmol	68 ± 35	79 ± 50	88 ± 38

RESULTS

Biochemical markers of bone resorption and cartilage degradation. IV pamidronate induced a rapid and sustained suppression of bone resorption and cartilage degradation for 12 days in both treatment groups (Figure 1). All 3 markers of bone resorption and the marker of cartilage degradation appeared to reach a lower nadir and a longer-lasting suppression in the 90 mg group compared to the 45 mg group, but these differences were not statistically significant. Rate of cartilage degradation over 12 days was significantly related to rate of bone resorption assessed by all 3 markers of bone resorption. Figure 2 shows the relationship between resorption and degradation illustrated by uNTx/Cr and uCTx-II/Cr levels before and for 12 days after a single pamidronate infusion.

WBR. The amount of pamidronate excreted in urine is shown in Figure 3. Renal excretion rate was high on day 1, after which the rate decreased exponentially over 12 days. The general pattern in both treatment groups appeared to be similar.

There was a remarkably wide range in the amount of BP excreted into urine and consequently retained on day 1 of treatment (WBR_1) in both treatment groups. There was no statistical difference in WBR_1 between the 90 mg and the 45 mg group when WBR_1 was expressed as a fraction of the administered dose (62.4 ± 14.9% vs 76.1 ± 12.1%, $p = 0.48$).

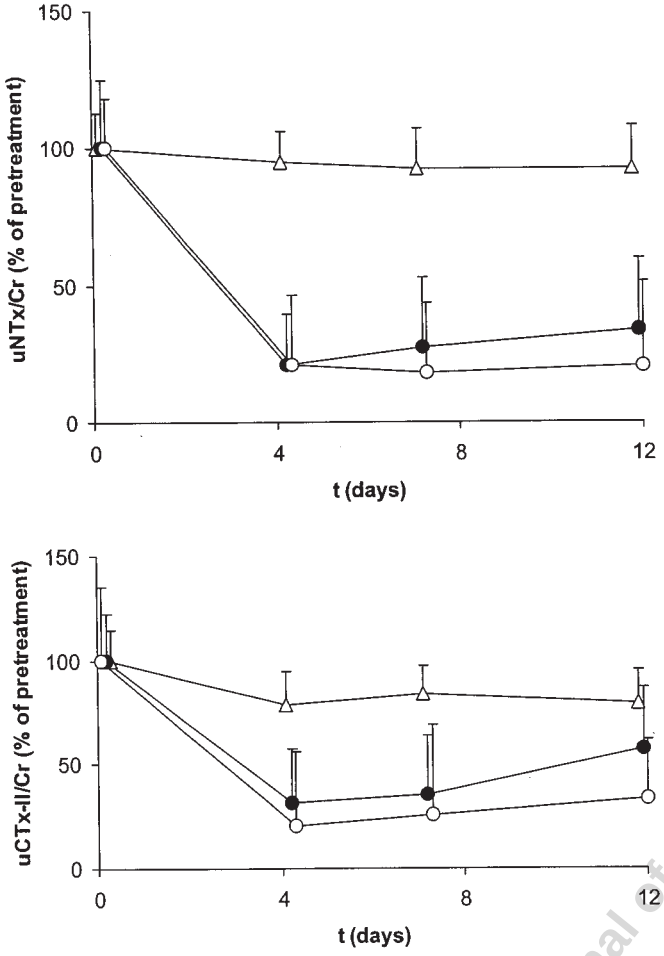

Figure 1. Mean (SEM) uNTx/Cr (A) and uCTx-II/Cr (B) for 12 days after IV administration of pamidronate in patients with rheumatoid arthritis (Δ placebo; ● 45 mg; ○ 90 mg).

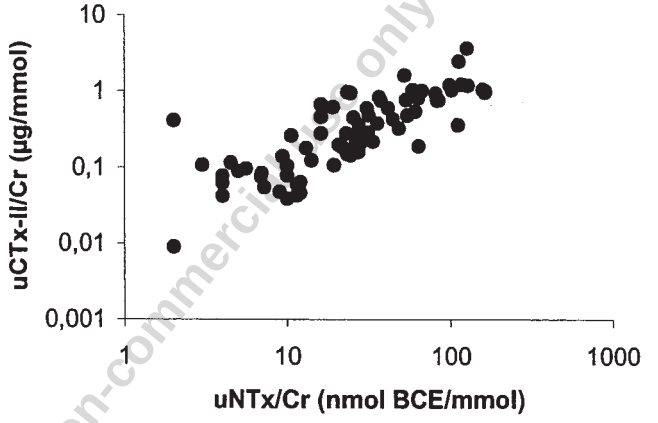

Figure 2. Relationship between rate of bone resorption (uNTx/Cr) and rate of cartilage degradation (uCTx-II/Cr) before and for 12 days after a single pamidronate infusion (45 or 90 mg) administered to 17 patients ($R^2 = 0.64$, p = 0.00, Pearson correlation coefficient on log-transformed data).

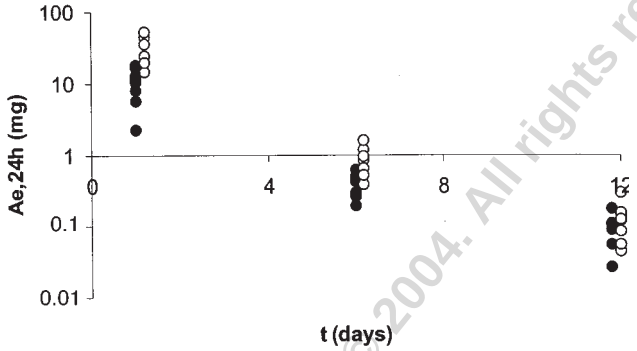

Figure 3. Amount of pamidronate excreted (Ae) into 24 h urine after IV administration of 45 mg (●) or 90 mg (○) pamidronate.

However, the absolute amount of pamidronate retained at day 1 in the 90 mg group was significantly higher than in the 45 mg group (56.3 ± 14 mg vs 34.1 ± 5.4 mg, $p < 0.01$). The range in WBR_1, however, was so wide that some of the patients who received 90 mg retained less BP (range 37–76 mg) than some patients who received 45 mg (range 27–43 mg). The half-lives (calculated from the elimination rate constants) were 3.0 and 2.2 days in the 45 and 90 mg group, respectively; these differences were not statistically significant. Elimination rate constants were 0.23 ± 0.14 once/day and 0.31 ± 0.14 once/day, respectively. Combined with the elimination rate, the overlap in WBR_t between groups was also seen during the remainder of the 12 days. The WBR_t according to the formula is shown for both treatment groups in Figure 4.

WBR_1 (expressed as percentage of dose) was not significantly related to creatinine clearance, prevalent (baseline) rate of bone resorption and cartilage degradation, or prevalent (baseline) disease activity (number of swollen joints and Ritchie articular index).

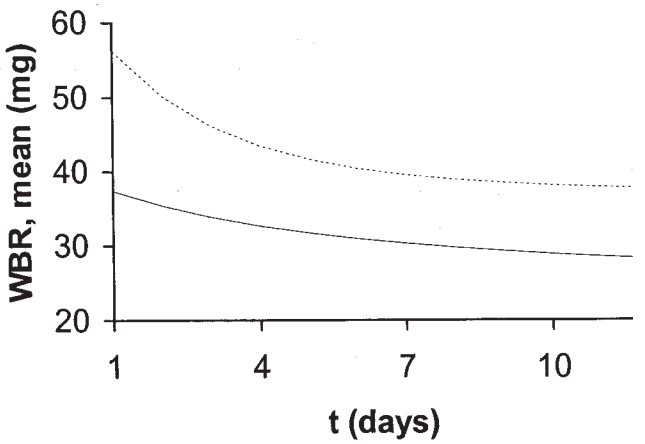

Figure 4. Whole body retention (WBR) of pamidronate after IV administration (calculated according to the formula) in patients who received 45 mg (——) and 90 mg (. . . . .).

Relationships between WBR and effect. There was a wide variation in rate of bone resorption before treatment, as shown for uNTx/Cr in Figure 5. In general, following treatment, WBR of pamidronate higher than 20 mg was associated with values within the range of premenopausal women, regardless of pretreatment rate of bone resorption. However, in patients with increased prevalent (baseline) bone resorption, there appeared to be a relationship between WBR and the level of suppression of bone resorption.

The results of uCTxII/Cr were very similar to those of the markers of bone resorption (Figure 6). There was a wide variation in values before treatment while, with retention exceeding 30 mg, all values were within the normal range. In patients with increased rate of cartilage degradation, there also appeared to be a relationship between WBR and the level of suppression of cartilage degradation.

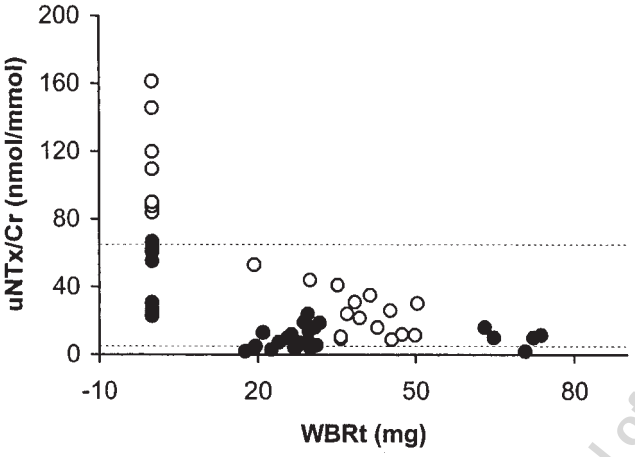

Figure 5. Relationship between whole body retention over time (WBR$_t$) of pamidronate and the rate of bone resorption, assessed by uNTx/Cr (. . . . normal range); ○ prevalent (baseline) rate of bone turnover outside normal range; ● prevalent range of bone turnover within normal range.

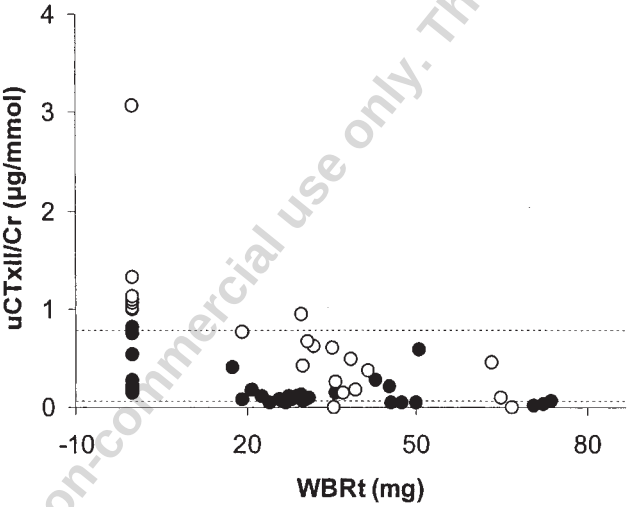

Figure 6. Relationship between WBRt of pamidronate and the rate of cartilage degradation, assessed by uCTx-II/Cr (. . . . normal range); ○ prevalent (baseline) rate of bone turnover outside normal range; ● prevalent (baseline) range of bone turnover within normal range.

DISCUSSION

Bone resorption markers are the most direct tool to determine the pharmacological effect of BP in patients, since inhibition of osteoclast-mediated bone resorption is the primary action of BP. After a single infusion of BP the suppression is fast, usually within a few days, and the suppression can be sustained for months depending on the dose and potency of the BP, as well as the disease activity of the primary disease.

Because BP enter the osteoclasts during bone resorption, the inhibition of bone resorption is influenced by the amount of BP at the surface of the bone[15]. After drug administration this amount changes with time, just as the suppression of bone resorption changes with time. Knowledge of the amount of BP in the skeleton and the relationship between this amount and the antiresorptive effect creates a pharmacological tool that can be used to design effective treatment regimens with IV bisphosphonate. BP also appear to have an effect on cartilage degradation[5,6,16], but this action is likely to be different both pharmacokinetically and pharmacodynamically. For example, BP bind selectively to hydroxyapatite and enter the osteoclasts during bone resorption[15], but it is not known how BP may affect cartilage degradation. Consequently, based on the specific pharmacology of BP, it is perhaps easier to assume relationships between the amount of BP at the skeleton and inhibition of bone resorption, than between this amount and inhibition of cartilage degradation. In osteoporosis we previously showed relationships between the amount of BP retained by the skeleton and inhibition of bone resorption, but these relationships are complex[14,17].

We show here that the amount of pamidronate retained in patients with active RA was comparable with the amount retained in patients with osteoporosis[14,17]. Both in RA and in osteoporosis, retention of the BP showed a wide range, but the average was similar in both diseases. This finding is consistent with previous studies by Fogelman, *et al*[22] and Steven, *et al*[23] using a ^{99m}Tc labeled bisphosphonate. This suggests that localized processes and localized binding of the BP in active RA do not contribute substantially to the total body retention of the BP, while it should be mentioned that with our methods we cannot discriminate between binding compartments. It may be that the relative distribution is different in patients with RA and osteoporosis. However, we did not find any relationship between either number of affected joints or disease activity and WBR. To address these issues more extensively, direct comparison between patients with RA and osteoporosis is required.

The wide variability in WBR of pamidronate was not related to renal function, most likely because the range of creatinine clearance of the patients was narrow. Despite the

fact that mean rate of bone resorption was higher than the range for premenopausal women, and variability was wide, a higher prevalent (baseline) rate of bone resorption was not associated with a higher WBR of the BP as was shown in patients with Paget's disease of bone[21].

The retention, however, was associated with suppression of bone resorption. A WBR higher than 20 mg was associated with normal levels of biochemical markers of bone resorption in all patients. Whether WBR lower than 20 mg might also be associated with suppression of bone resorption cannot be derived from our data as the doses of pamidronate used, and concomitant body retentions, were at the flat part of the concentration effect curve. However, especially in the patients with high prevalent (baseline) rate of bone resorption, there appeared to be an association between WBR and suppression of bone resorption (Figure 5).

As with bone resorption, the rate of cartilage degradation was also associated with WBR of the BP. Suppression of cartilage degradation into the normal range was associated with a WBR higher than 30 mg, while in patients with a prevalent (baseline) rate of cartilage degradation higher than the normal range, a higher WBR was associated with an increased suppression of cartilage degradation.

Although the relative binding to periarticular sites and those at the rest of the skeleton could not be determined by the method used, it can be concluded that an increase in WBR of BP is associated with a higher decrease of bone resorption and cartilage degradation. It may therefore be that these pharmacokinetic/pharmacodynamic relationships are responsible for the differences in clinical responses in RA[2-4,10], although we did not find a relationship between WBR and clinical response (number of affected joints, Ritchie score, and DAS). This could be explained by the relatively short period of observation. Future studies investigating these relationships should incorporate a smaller, less effective dose, and should also have a longer period of observation in order to investigate the resolution of the inhibitory effect too, which is extremely important for pharmacokinetic/pharmacodynamic studies.

The question remains whether the BP effect on cartilage degradation is direct or indirect. The CTx-II marker we measured has been shown to be a specific marker of cartilage degradation in patients with osteoarthritis[19]. On the other hand, degradation of cartilage may be induced by cytokines that are also released during bone remodelling[20]. This could be especially relevant during active RA where active bone sites are near to cartilage, and local interaction processes between cartilage destruction and bone resorption are likely to play an important role in the pharmacology of BP in active RA[20]. Inhibition of cartilage degradation could therefore be a result of inhibition of bone resorption, a thesis supported by *in vitro* results in bone explants (Ermond van Beek, *et al*, unpublished observations) in which inhibition of cartilage degradation follows the inhibition of bone resorption. In our study, rate of cartilage degradation was related to rate of bone resorption, but a time lag between decrease of bone resorption and decrease of cartilage degradation could not be observed, possibly due to the rapid responses of the markers and the relatively low sampling frequency.

In conclusion, this is the first study in which the effect of BP treatment in RA has been studied in relation to the amount of BP retained by the body. This amount is similar to that reported for osteoporosis, suggesting that the local process in active RA does not lead to higher retention of BP. The variability in BP retention and its relationship to the effect suggest that therapeutic regimens based on BP retention rather than on dosing may help to resolve the current discrepancies concerning the effectiveness of BP therapy in patients with RA.

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