Prolonged bisphosphonate release after treatment in women with osteoporosis. 
Relationship with bone turnover

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Abstract

Bisphosphonates (BP), especially alendronate and risedronate, are the drugs most commonly used for osteoporosis treatment, being incorporated into the skeleton where they inhibit bone resorption and are thereafter slowly released during bone turnover. However, there are few data on the release of BP in patients who have received treatment with these drugs for osteoporosis. This information is essential for evaluating the possibility of BP cyclic therapy in these patients and for controlling their long-term presence in bone tissue. This study evaluated the urinary excretion of alendronate and risedronate in patients treated with these drugs for osteoporosis and analysed its relationship with bone turnover, time of previous drug exposure and time of treatment discontinuation. We included 43 women (aged 65 ± 9.4 years) previously treated with alendronate (36) or risedronate (7) during a mean of 51 ± 3 and 53 ± 3 months, respectively, who had not been treated with other antiosteoporotic treatment and with a median time of discontinuation of 13.5 and 14 months, respectively. Both BP were detected in 24-hour urine by HPLC. In addition, bone formation (PINP) and resorption (NTx) markers were analysed. Both BP were also determined in a control group of women during treatment. Alendronate was detected in 41% of women previously treated with this drug whereas no patient previously treated with risedronate showed detectable urinary values. All control patients showed detectable values of both BP. In patients with detectable alendronate levels, the time of drug cessation was shorter than in patients with undetectable values (12 [6–19] versus 31 [7–72] months, p = 0.001). Alendronate was not detected in any patient 19 months after treatment cessation. Alendronate levels were inversely related to time of treatment discontinuation (r = −0.403, p = 0.01) and the latter was directly related to NTx (r = 0.394, p = 0.02). No relationship was observed with age, length of drug exposure, renal function or weight.

In conclusion, contrary to risedronate, which was not detected in patients after cessation of treatment, alendronate was frequently detected in women previously treated with this agent up to 19 months after discontinuation of therapy. The relationship between alendronate levels and bone resorption and time of treatment cessation further indicates a residual effect of this drug in bone, despite treatment discontinuation.

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Introduction

Bisphosphonates (BP), especially alendronate and risedronate, are the drugs most commonly used for the treatment of osteoporosis. These compounds are rapidly cleared from the circulation with nearly 20–50% of the dose administered being incorporated into the skeleton, where it inhibits bone resorption, and the remainder is excreted in urine [1,2]. After their incorporation into bone tissue, BP are slowly released from bone by means of the osteoclast during the bone resorption process. Thus, pharmacokinetic studies have previously estimated a 10-year mean life for a BP such as alendronate [3], with a much shorter mean life for risedronate [4]. However, with the exception of a recent study that detected the presence of pamidronate in urine up to 7 years after its administration in children previously treated with this drug [5], at present, there are no data on the release of such compounds in patients who have followed treatment with these drugs for osteoporosis. This information could be a determinant to evaluate the possibility of cyclic therapy as well as the duration of “drug holidays” and to control the long-term presence of these drugs in bone tissue, especially in young women, in whom the possibility of future pregnancies limits the use of these drugs.

Therefore, the aim of the present study was to evaluate the urinary excretion of alendronate and risedronate in patients who have been previously treated with these drugs for osteoporosis and to analyse their relationship with bone turnover.

Patients and methods

We performed a cross-sectional study including 43 women (40 postmenopausal) aged 36–80 years (mean age ± standard deviation [SD] 65 ± 9.4 years) followed in the Metabolic Bone Diseases Unit of...
our department, who had been previously treated with alendronate (either 10 mg/day or 70 mg/weekly) (n = 36) or risedronate (either 5 mg/day or 35 mg/weekly) (n = 7) for osteoporosis and had not received other antosteoporotic treatment (except for calcium and vitamin D supplements) up to the time of the analysis.

Weight, height, time of previous drug exposure, time of treatment cessation (time between cessation of treatment and urinary BP determination) and renal function were recorded in all patients. The median time of treatment discontinuation was 13.5 (range 6–72) and 14 (range 5–60) months for alendronate and risedronate, respectively. In addition, coinciding with BP determination, bone turnover parameters were analysed using bone formation (PINP) and bone resorption (NTx) markers. Both BP were also determined in urine in a control group of 7 women during alendronate (70 mg/weekly) or risedronate (35 mg/weekly) treatment, with a mean duration of treatment of 47.3 ± 25 months (range 24–92 months).

Informed consent was obtained from all the subjects, and the study was approved by the Ethics Committee of the Hospital Clinic of Barcelona.

Detection of bisphosphonates and biochemical determinations

Alendronate and risedronate concentrations were detected in 24-hour urine by high performance liquid chromatography (HPLC) with UV diode array detection for risedronate and fluorescence detection for alendronate. Urine samples were frozen and stored at −20 °C for subsequent BP determination.

For alendronate determination we used an HPLC method that involves diethylamine (DEA) solid-phase extraction (SPE), 9-fluorenylmethyl derivative (FMOC) and fluorescence detection. Sample preparation included protein precipitation with trichloroacetic acid (TCA), triple co-precipitation of alendronate with calcium phosphate in alkaline conditions and derivatization with 9fluorenylmethyl chloroformate in citrate buffer at pH 11.9 (Sparidans et al. [6] and Yun et al. [7]). For risedronate determination, we used an ion-pair HPLC method employing ion-pair solid phase extraction and UV detection. Sample preparation involved triple co-precipitation of risedronate with calcium phosphate in alkaline conditions and ion-pair solid extraction (Hui-Jia et al. [8] and Vallano et al. [9]). The sensitivity for urinary detection with these procedures was 2 ng/ml and 6 ng/ml for alendronate and risedronate, respectively. These limits were defined as a BP concentration with a peak ≥ three-fold the height of the chromatographic baseline. The assays were highly specific, and no endogenous interferences were encountered during the procedure. Potential interferences with these BP were investigated assaying the chromatographs being found to be free of interfering peaks at the retention time of both BP.

The inter- and intraday variability ranged from 0.085 to 0.097 between series and from 0.053 to 0.079 within series.

In addition, blood and second morning urine samples were obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast to determine serum creatinine, calcium and phosphate, as well as the obtained between 8:00 and 10:00 a.m. after an overnight fast.

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Statistical analysis

Means and standard deviations were used to summarise continuous variables, and variables not normally distributed were reported as medians and ranges. Both parametric and, when the data were not normally distributed, non-parametric statistical methods were used to analyse the study data. The Student’s t-test and the non-parametric Kruskal–Wallis test were used to compare differences for continuous variables. Chi-square was used to test the differences between proportions. Correlations by Pearson test and multiple linear regression analysis were used to evaluate the relationship between variables. A p value of <0.05 was considered statistically significant. Statistical analyses were conducted using the SPSS version 16.0.

Results

The characteristics of the patients are shown in Table 1. Except for urinary BP detection, no significant differences were observed between patients previously treated with alendronate or risedronate, with a similar time of previous drug exposure and drug discontinuation. Thus, alendronate was detected in 41% of women who had been previously treated with this drug whereas no patient receiving previous treatment with risedronate showed detectable urinary values of this BP, even in patients analysed 5 months after risedronate discontinuation (2 cases). All control group patients showed detectable values of both types of BP. Nevertheless, the mean urinary values of alendronate in patients during alendronate treatment were much higher than those observed in the patients who had been previously treated with this drug (Table 2). On comparing patients previously treated with alendronate with detectable versus undetectable levels (Table 3), no other significant differences were observed except for a shorter time of drug cessation in those with undetectable values of this BP [12 range, 6–19] versus [1] range, 7–72] months, p = 0.001). Thus, bone turnover markers, renal function, and time of previous drug exposure were similar in the two groups of patients (Table 3). Interestingly, no patient with more than 19 months since cessation of alendronate treatment showed detectable values of the drug in urine, whereas most patients (67%) with a shorter time since cessation showed detectable values.

In addition, we observed an inverse correlation between alendronate levels and time of treatment discontinuation (r = −0.403, p = 0.01). Time of discontinuation was also directly related to the bone resorption marker NTx (r = 0.394, p = 0.02) (Fig. 1). Conversely, no relationship was observed between alendronate levels and age, length of drug exposure, renal function parameters or weight. On linear regression analysis, the time of drug discontinuation (p = 0.0032) and urinary alendronate levels (p = 0.03) were the principal factors related to urinary NTx levels. When only patients with detectable urinary levels of alendronate were evaluated no relationship was observed between alendronate concentrations and the time of drug discontinuation or the NTx values.

Discussion

The results of this study show that alendronate is frequently detected in women previously treated with this agent for osteoporosis.

Table 1

<table>
<thead>
<tr>
<th>Characteristics of the patients (data are mean ± SD).</th>
<th>Alendronate (n = 36)</th>
<th>Risedronate (n = 7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.7 ± 10</td>
<td>62.7 ± 5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Length of treatment (months)*</td>
<td>50.9 ± 30.9</td>
<td>53.2 ± 32</td>
<td>n.s.</td>
</tr>
<tr>
<td>Time of discontinuation (months)*</td>
<td>13.5 (6–72)</td>
<td>14 (5–60)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.78 ± 0.16</td>
<td>0.79 ± 0.14</td>
<td>n.s.</td>
</tr>
<tr>
<td>GFR (ml/min)</td>
<td>80.7 ± 20.5</td>
<td>81.1 ± 23</td>
<td>n.s.</td>
</tr>
<tr>
<td>PINP (ng/ml)*</td>
<td>36.5 (15–198)</td>
<td>43 (17–82)</td>
<td>n.s.</td>
</tr>
<tr>
<td>NTx (NM/mM)</td>
<td>45.5 ± 18</td>
<td>41.7 ± 18</td>
<td>n.s.</td>
</tr>
<tr>
<td>Detection of BP in urine (%)</td>
<td>41</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

GFR: glomerular filtration rate; PINP: procollagen type I N propeptide; NTx: N-terminal cross-linked telopeptide of type I collagen.

* Variables that are not normally distributed are reported as median (range).
In addition, we observed a relationship between alendronate levels and both bone resorption and time of treatment cessation further suggesting a residual effect of this drug in bone, despite treatment discontinuation. Conversely, no patient previously treated with risedronate had detectable values of the drug after discontinuation of therapy.

Alendronate was detected in 41% of postmenopausal women previously treated with this agent, being observed up to 19 months after discontinuation of therapy. Indeed, the time of drug discontinuation was the principal factor related to the detection of this agent in urine. However, no relationship was observed with other factors previously related to the long term effect of this drug such as the length of therapy or renal function parameters [1,2,11,13]. Nonetheless, it should be noted that most of our patients had a normal GFR, none being lower than 40. Our results corroborate previous findings indicating a residual effect of BP, especially alendronate, in bone after treatment cessation. Thus, postmenopausal women treated with this agent for 5 years seem to maintain bone mass and bone turnover up to 2 years after cessation of therapy [14–16], thereby suggesting that the skeletal retention of BP might contribute to their prolonged duration effect when treatment is stopped. Interestingly, this period of time of approximately 2 years coincides with the period of detection of this BP in urine in our study and is also in agreement with the period of detection of this agent after a single intravenous administration [3]. In addition, we observed a relationship between the bone resorption marker, NTx, and urinary levels of alendronate, further supporting this hypothesis.

Conversely, risedronate was not detected in any patient after cessation of treatment, independently of the time of cessation of therapy. It could be argued that the sensitivity of the detection method differs for the two drugs. However, both BP were detected in control patients and also had a similar lower limit of detection thereby not supporting this hypothesis. Indeed, we were not able to detect risedronate even 5 months after treatment cessation. These data are in accordance with previous clinical findings related to this agent [17]. Thus, contrary to alendronate treated patients those treated with risedronate normally began to lose bone and increase bone resorption 1 year after cessation of treatment [18,19], thus suggesting a less prolonged retention of this agent in bone.

All these results may be explained in part by the differences in the specific pharmacological properties of the two BP, such as their selective uptake at active bone sites, suppression of osteoclast-mediated bone resorption, and long-term skeletal retention [1,2]. All these properties depend on the structure of the BP molecule, resulting in great differences in their antiresorptive potency and in the binding affinity to hydroxyapatite, the latter being related to longer retention in bone. Thus, alendronate has shown higher binding affinity to bone mineral than risedronate [11,17], which may explain our findings. Indeed, in a recent study one of the BP with the greatest bone retention such as zoledronic acid showed a 3-year antiresorptive effect after one isolated intravenous infusion [20].

As previously commented, approximately 20–50% of BP entering the circulation is retained by the skeleton and the rest is excreted unmodified in the urine, thereby making this the best medium to detect these agents. However, the proportion of BP retained by bone seems to be related to skeletal turnover [1,11,21,22]. Unfortunately, we could not compare the baseline bone turnover previous to initiating therapy in our patients, a factor that has also been related to the long term effect of these drugs. Although other factors such as the adherence to both treatments in our patients was not evaluated, indirect data in patients with similar characteristics in our unit have demonstrated similar adherence to the two BP [23].

Our findings could be applied to clinical practice. Thus, in view of the present results and also in accordance with previous data, alendronate could theoretically be used as a cyclical therapy for treatment of osteoporosis, especially in postmenopausal women and men. On the other hand, risedronate should be used as continuous therapy or with a shorter period of discontinuation, and is probably the BP of choice, if needed, in young patients because of its lower skeletal retention.

This study does have several limitations: the use of different HPLC methodologies for detection of each of the BP, a limitation that is linked to the different characteristics of the compounds; the cross-section study design; the absence of data on pre-treatment bone turnover, which is almost certainly related to levels of bone turnover after drug cessation; the absence of data on compliance in individuals previously treated with both BP and the small sample size of the risedronate group. The latter however, could be partly explained by the methodology of our study, in that patients were recruited if they had been previously treated with one of the two study drugs and had not taken other antosteoporotic therapy up to the time of analysis. Since patients receiving risedronate often require antosteoporotic treatment after discontinuation of this BP, few patients who had not done so could be found and thereafter be included in the study. In addition, at the time of this study alendronate was more frequently used for osteoporosis treatment.

In conclusion, contrary to risedronate, which was not detected in patients after cessation of treatment, alendronate was frequently...
detected in women previously treated with this agent up to 19 months after discontinuation of therapy. The relationship between alendronate levels and both bone resorption and time of treatment cessation further indicates a residual effect of this drug in bone, despite treatment discontinuation. These findings may allow the optimization of treatment regimens with these BP in osteoporosis.

References