AMINOHEXANE BISPHOSPHONATE SUPPRESSES BONE TURNOVER IN POSTMENOPAUSAL WOMEN MORE RAPIDLY THAN OESTROGEN-GESTAGEN THERAPY

J. H. TOBIAS, C. J. LAVERSUCH, T. J. CHAMBERS* and A. C. GALLAGHER*
Osteoporosis Screening Unit and *Department of Histopathology, St George's Hospital Medical School, London SW17 0RE

SUMMARY

Although animal studies suggest that there may be major differences between the effects of bisphosphonates and ovarian hormones on skeletal metabolism, whether this also holds for their actions in patients is unknown. To address this question, we compared the effects of 12 weeks treatment with HRT on bone turnover markers in osteopenic postmenopausal women with those of an amino-bisphosphonate. Women within 15 yr of the menopause, with a lumbar and/or femoral neck bone mineral density 1 s.d. below the predicted value, received either oestradiol valerate 2 mg and dydrogesterone 5 mg (E/D; n = 16) or aminohexane bisphosphonate 400 mg (AHBP; n = 9). Urine and serum samples were collected on two separate occasions before starting treatment, and 1, 2, 4, 8 and 12 weeks afterwards. To assess bone resorption, we measured the urinary deoxypyridinoline/creatinine ratio (DPD/crea), while serum alkaline phosphatase (ALP), osteocalcin and C-terminal propeptide of type 1 collagen (CICP) were analysed to assess bone formation. Repeated measures analysis of variance revealed a highly significant decrease in DPD/crea over the treatment period. Furthermore, this pattern of response differed significantly between the two treatment groups, since DPD/crea was maximally suppressed within 2 weeks of starting AHBP, while E/D showed little decrease until 8 weeks. AHBP was also found to suppress ALP, osteocalcin and CICP more rapidly than E/D, the former reducing these markers by 8 weeks, while E/D caused little inhibition even by 12 weeks. We conclude that, in the doses used in this study, AHBP appears to suppress bone turnover significantly more rapidly than E/D, suggesting that clinically important differences may exist in the effects of bisphosphonates and ovarian hormones on bone metabolism.

KEY WORDS: Bisphosphonate, Oestrogen, Bone turnover, Postmenopausal women.

The last decade has seen a great increase in the number of therapies available for treating osteoporosis. In addition, the widespread availability of DXA scanners for monitoring bone mass provides an alternative means of assessing the therapeutic response to simply waiting to see whether fractures occur. However, since serial bone mass measurements need to be taken at least 12 months apart to detect significant bone loss, other methods are required for investigating early responses. Since many agents used to treat osteoporosis are thought to act by suppressing bone resorption, like hormone replacement therapy (HRT) [1-3] and bisphosphonates [4], one approach to the early detection of responses to these agents is to measure their effect on bone turnover markers. HRT and bisphosphonate therapy have both been documented to suppress a number of bone formation and resorption markers within 12 weeks of starting treatment [5-10], suggesting that this may indeed be a useful way of monitoring the early response of patients to bone-sparing agents. However, since HRT and bisphosphonates are thought to influence skeletal metabolism through distinct mechanisms, important differences may exist between their effects on bone markers, in which case it may be necessary to develop treatment-specific strategies. For example, while bisphosphonates have been found to suppress bone formation markers following relatively short periods of treatment in postmenopausal women [5], this may not occur following treatment with certain HRT regimes [11]. However, to our knowledge, there has been no previous attempt to compare directly the effects of HRT and bisphosphonates on bone turnover markers in postmenopausal women. Therefore, to determine whether important differences do indeed exist between the early effects of HRT and bisphosphonates on bone turnover markers in postmenopausal women, we compared their effects on bone markers over a 12 week period.

METHODS

Protocol approval was obtained from the St George's Hospital ethics committee. Women were recruited from patients referred to the St George's Osteoporosis Screening Unit for bone densitometry. Subjects were deemed suitable for inclusion if they were within 15 yr of the menopause, and their bone mineral density was found to be 1 s.d. or more below the predicted value at the lumbar spine (L2-4) and/or femoral neck. Women were excluded if they had received HRT within the last 6 months, had ever been treated with bisphosphonates or oral steroids, or if there was evidence on baseline biochemical analysis of osteomalacia, hyperthyroidism or hyperparathyroidism. No one included in the study had previously sustained a vertebral fracture, as evidenced by
pre-treatment spinal X-ray, which was performed on each subject.

Women who agreed to participate in the study were asked to state their preference for treatment with either bisphosphonate or hormone replacement, following a discussion of the relative advantages and disadvantages of these therapies in the treatment of osteoporosis. Written informed consent was subsequently taken, after which subjects were commenced on their preferred treatment, which was continued for 12 weeks. Hormone replacement was given as oestradiol valerate 2 mg daily (Sandoz Pharmaceuticals, Frimley, Surrey) and dydrogesterone 5 mg daily (Duphar Laboratories, Southampton, Hants) (E/D; n = 16), and bisphosphonate as aminohexane bisphosphonate 400 mg daily (a gift from Gentili S.P.A., Pisa, Italy) (AHBP; n = 9). The latter is an orally administered aminobisphosphonate of proven clinical efficacy in the treatment of Paget's disease at this dose [12-14].

Twenty-five subjects were recruited to the study over a 12 month period. The two treatment regimes were subsequently found to be generally well tolerated. However, two patients in the E/D group discontinued treatment after 4 weeks: one due to the development of non-specific musculoskeletal symptoms and one due to the exacerbation of pre-existing hypertension. One patient in the AHBP group withdrew after 2 weeks due to the occurrence of gastrointestinal side-effects.

Two-hour urine collections, commenced after the first morning void, and serum samples were obtained on two separate occasions 1 week apart prior to the start of therapy, and 1, 2, 4, 8 and 12 weeks afterwards. Urine samples were stored in multiple aliquots at -20°C. Venesection was performed at the same time of day for each subject and serum subsequently stored in multiple aliquots at -70°C. Urinary creatinine (crea) and serum calcium were measured on a multichannel analyser, the latter being corrected to a serum albumin concentration of 42 g/l. PTH was measured by radioimmunoassay (Department of Medical Biochemistry, University Hospital of Wales, Cardiff).

To assess bone resorption, urinary excretion of free deoxypyridinoline cross-links (DPD) was measured by ELISA (Pyrilinks-D, Metra Biosystems Inc., Pala Alto, CA, USA) [15], results being expressed as a ratio to urinary creatinine excretion. To avoid any degradation of cross-links caused by light exposure, samples remained shielded from light throughout. Serum levels of bone formation markers, which consisted of the bone isoenzyme of alkaline phosphatase (ALP), the C-terminal propeptide of type I collagen (CICP) and osteocalcin, were also measured by an ELISA method (Metra Biosystems) [16, 17]. To limit inter-assay variation, samples from a given patient were analysed in the same assay run. To obtain normal reference data, we also analysed these markers in 16 healthy premenopausal female volunteers (mean age 35 yr). In our hands, intra-assay variability for these ELISAs was <8%.

Baseline characteristics of subjects in the two treatment groups were compared by unpaired Student's t-test. To compare the effect of E/D and AHBP on the above parameters, each result was divided by the mean of the two pre-treatment values and multiplied by 100, giving the percentage of mean baseline value. Results were expressed as mean ± s.d. We subsequently performed a repeated measures analysis of variance on bone marker data from subjects who had completed the study, and recorded whether there was a statistically significant effect of time, treatment group, or an interaction between these (Statview 4.0, Abacus Concepts, Cupertino, CA, USA).

**RESULTS**

Despite our use of a non-randomized study design, there were no significant differences in baseline patient

### TABLE I

<table>
<thead>
<tr>
<th>Baseline patient data</th>
<th>E/D (n = 16)</th>
<th>AHBP (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>53.9 ± 5.3</td>
<td>56.9 ± 4.9</td>
</tr>
<tr>
<td>Age of menopause</td>
<td>47.1 ± 5.5</td>
<td>48.9 ± 2.0</td>
</tr>
<tr>
<td>Years since menopause</td>
<td>6.8 ± 3.9</td>
<td>7.9 ± 4.6</td>
</tr>
<tr>
<td>Lumbar spine BMD Z score</td>
<td>-1.6 ± 1.0</td>
<td>-1.3 ± 0.8</td>
</tr>
<tr>
<td>Femoral neck BMD Z score</td>
<td>-1.0 ± 0.6</td>
<td>-1.0 ± 0.6</td>
</tr>
<tr>
<td>Corrected calcium (nmol/ml)</td>
<td>2.30 ± 0.09</td>
<td>2.22 ± 0.11</td>
</tr>
<tr>
<td>PTH</td>
<td>3.7 ± 1.5</td>
<td>5.1 ± 2.3</td>
</tr>
<tr>
<td>DPD/crea (nmol/mmol)</td>
<td>7.3 ± 1.5</td>
<td>7.5 ± 3.0</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>10.7 ± 4.1</td>
<td>8.4 ± 3.1</td>
</tr>
<tr>
<td>ALP (nmol)</td>
<td>22.1 ± 6.7</td>
<td>18.7 ± 4.9</td>
</tr>
<tr>
<td>CICP (ng/ml)</td>
<td>122 ± 46</td>
<td>125 ± 54</td>
</tr>
</tbody>
</table>

Results show the mean ± s.d. All subjects were Caucasian, other than one Asian subject in the AHBP group. Reference data (mean and interquartile range), obtained from 16 normal premenopausal women, were as follows: 5.3 (2.4-7.7) nmol/mmol for DPD/crea ratio, 8.2 (7.7-11.5) ng/ml for osteocalcin, 14.8 (16.0-27.1) nmol/ml for ALP and 123 (100-145) ng/ml for CICP. BMD is bone mineral density.

### TABLE II

<table>
<thead>
<tr>
<th>Serum calcium and PTH responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium</td>
</tr>
<tr>
<td>E/D</td>
</tr>
<tr>
<td>100 ± 2</td>
</tr>
<tr>
<td>AHBP</td>
</tr>
<tr>
<td>100 ± 3</td>
</tr>
<tr>
<td>PTH</td>
</tr>
<tr>
<td>E/D</td>
</tr>
<tr>
<td>100 ± 17</td>
</tr>
<tr>
<td>AHBP</td>
</tr>
<tr>
<td>100 ± 15</td>
</tr>
</tbody>
</table>

Results show the percentage of mean baseline PTH and adjusted calcium (mean ± s.d.) for women treated with E/D or AHBP for 12 weeks.
Figure 1.—Results show the percentage of mean baseline DPD/creatinine ratio (mean ± S.D.), for women treated with E/D or AHBP for 12 weeks. Repeated measures analysis of variance revealed a significant effect of time (P = 0.001), and a significant interaction between time and treatment group (P = 0.04) (P = 0.4 for the overall effect of treatment group).

Characteristics between women receiving E/D and AHBP (Table I). Mean values in our postmenopausal study population were within the interquartile range of our premenopausal reference sample. A minor hypocalcaemic action of E/D was observed, associated with a small compensatory increase in parathyroid hormone (PTH), although no such change was evident following AHBP administration (Table II).

DPD/crea showed a highly significant decrease over the treatment period, the pattern of which differed significantly in the two treatment groups, as evidenced by a significant interaction between time and treatment group (Fig. 1). This difference reflected a more rapid reduction in DPD/crea following the administration of AHBP, which caused maximal suppression within 2 weeks of starting therapy, in contrast to E/D, which showed little tendency to reduce DPD/crea until treatment had been given for 8 weeks.

Serum ALP showed a highly significant reduction following treatment, the pattern of which was also different between the two treatment groups, as judged by the highly significant interaction between time and treatment group (Fig. 2). Once again, this difference was characterized by a more rapid suppression after treatment with AHBP: the latter decreased serum ALP by 8 weeks, which was sustained at 12 weeks, while E/D had little effect on ALP at 8 weeks and caused a minor decrease only at 12 weeks. In addition, both treatments were associated with a minor, transient decrease in ALP after 2 weeks.

We also found a significant effect of treatment on serum osteocalcin, which appeared to consist of two distinct components (Fig. 3). Firstly, AHBP and E/D both caused an initial, transient increase in serum osteocalcin. Secondly, longer periods of treatment were associated with a reduction in serum osteocalcin. However, while AHBP reduced serum osteocalcin by 8 weeks, an inhibitory effect of E/D was not evident until 12 weeks, this difference presumably being the basis for the significant interaction between time and treatment group which we observed. Treatment was also found to influence serum CICP differently according to treatment group, as AHBP suppressed serum CICP by 8 weeks, which was sustained at 12 weeks, while E/D had little effect throughout (Fig. 4).

DISCUSSION

We have found that AHBP administration suppresses urinary excretion of the resorption marker DPD/crea considerably more rapidly than treatment with E/D. Moreover, after treatment for 8 weeks, the inhibitory effect of AHBP on bone resorption led to a reduction in serum levels of all those bone formation markers analysed (i.e. ALP, osteocalcin and CICP), while E/D had relatively little effect on these. Taken together, these observations suggest that, at the doses used in this study, AHBP suppressed bone turnover more rapidly than E/D. Whether this difference holds true for other bisphosphonate and HRT regimes is currently unclear; to our knowledge, this question has not been previously addressed by directly comparing the effects of other bisphosphonate
TOBIAS ET AL.: BISPHOSPHONATE AND HRT EFFECTS ON BONE TURNOVER

639

Results show the percentage of mean baseline osteocalcin value (mean ± s.d.), for women treated with E/D or AHBP for 12 weeks. Repeated measures analysis of variance revealed a significant effect of time (P = 0.002), and a significant interaction between time and treatment group (P = 0.02) (P = 0.6 for the overall effect of treatment group).

Fig. 4.—Results show the percentage of mean C1CP value (mean ± s.d.), for women treated with E/D or AHBP for 12 weeks. Repeated measures analysis of variance revealed a significant effect of time (P = 0.05), and a significant interaction between time and treatment group (P = 0.01) (P = 0.4 for the overall effect of treatment group).

Several previous studies have examined the early effects of various HRT and bisphosphonate regimes on bone turnover markers separately, the results of which are broadly consistent with our present findings. For example, other HRT regimes have been found to reduce urinary DPD/crea after treatment for 6 [7] and 12 weeks [10, 18]. Our finding that E/D did not appear to reduce serum ALP until 12 weeks, apart from a transient decrease at 2 weeks, is also consistent with previous reports. For example, oestradiol combined with norethisterone has been found to reduce total and bone ALP after treatment for 3 and 4 months, respectively [8, 9], while oestrogen given alone or in combination with norethisterone had little consistent effect after shorter periods of treatment [6, 7, 11].

In contrast, previous studies examining the short-term effect of other HRT regimes on serum levels of osteocalcin had led to conflicting results. For example, while HRT has been found to have little suppressive effect on osteocalcin levels during the first 12 weeks of treatment [9, 11], others have found that serum osteocalcin is reduced within this period [6-8]. Interestingly, of the three studies which found HRT to suppress rapidly serum osteocalcin, oestrogen was given alone in two [6, 7], whereas in the two previous investigations where no significant suppression was seen [9, 11], as in the present study, oestrogen was also combined with a gestagen. This raises the possibility that gestagens modify the short-term effect of HRT on serum osteocalcin, consistent with reports which suggest that progesterone may stimulate osteoblast activity [19-22].

Our observation that AHBP rapidly suppressed bone resorption, as judged by DPD/crea, followed after a delay of several weeks by a decrease in serum markers of bone formation, is consistent with results of a recent study of the amino-bisphosphonate alendronate. In the latter, oral alendronate was found to suppress urinary collagen cross-link excretion in postmenopausal women after treatment for 3 weeks, while serum ALP and osteocalcin were reduced at 6 weeks [5]. However, recent evidence suggests that ELISA methods which measure free collagen cross-links, as used in the present study, may underestimate the true effect of bisphosphonate therapy on bone resorption. For example, oral alendronate and i.v. disodium pamidronate (APD) have recently been reported to reduce the excretion of peptide-bound cross-links in osteoporotic patients, but to have little effect on free cross-link excretion [23, 24].

Recent observations also suggest that assays which measure free DPD may be more sensitive at detecting responses to HRT [24]. Therefore, although we found that AHBP and E/D were associated with similar DPD/crea values after treatment for 8 weeks, it is possible that the former had nevertheless caused more marked resorption suppression. If AHBP is indeed a more potent anti-resorptive than E/D, this might also explain why the former appeared to suppress bone
The expected to result in a delayed onset of bone resorption. As an example, ovariectomy has been found to enhance osteoclast activity in vivo. In contrast, bisphosphonates appear to suppress bone resorption through a relatively rapid action on actively resorbing osteoclasts. This suggests that sex steroids may function to modulate the activity of osteoclast precursors, which might be expected to result in a delayed onset of bone resorption inhibition. In contrast, bisphosphonates appear to suppress bone resorption through a relatively rapid action on actively resorbing osteoclasts.

In addition to the suppression of bone turnover markers by E/D and AHBP, these agents transiently increased serum osteocalcin within the first few weeks of starting treatment. A similar rise in serum osteocalcin has been reported after i.v. APD administration in patients with Paget's disease. In addition, oestrogen-gestagen therapy has been found to cause a small increase in osteocalcin over the first weeks of treatment, although no such rise was seen after oestrogen alone. A possible explanation for this transient increase in serum osteocalcin is that anti-resorptive therapies may suppress the serum calcium concentration, leading to a compensatory increase in PTH which may directly stimulate osteoblast activity. However, in this study, only minor changes in serum PTH following treatment were observed. Alternatively, these agents might exert a direct stimulatory effect on osteoblast activity, as suggested by certain in vitro studies.

In conclusion, we have found that, in the doses used in this study, treatment with AHBP suppressed bone turnover in postmenopausal women more rapidly than E/D. Whether this difference holds for other HRT and bisphosphonate regimes is unclear, since, to our knowledge, no previous study has directly compared the early effects of bisphosphonates and HRT on bone turnover markers in this group. However, to the extent that previous clinical and laboratory studies are consistent with our findings, we suggest that testosterone may be capable of detecting the response of postmenopausal women to anti-resorptive agents considerably earlier than those commencing treatment with bisphosphonates rather than HRT.

**Acknowledgements**

JHT was supported by the Arthritis Research Council and CJL by a project grant from Shire Pharmaceuticals.

**References**


18. Hassager C, Colwell A, Assiri AMA, Eastell R, Russell RGG, Christiansen C. Effect of menopause and hormone replacement therapy on urinary excretion of pyridinium...


