

PTH and interactions with bisphosphonates

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Abstract

We report that a therapeutic dose of the antiresorptive bisphosphonate alendronate administered to skeletally mature rats for the duration of 16 weeks significantly blunted the anabolic response to a high dose SDZ PTS 893 in the tibia and femur but not in lumbar vertebra. Effects were seen at the level of bone mass (DEXA, pQCT) as well as in biomechanical tests. In one arm of this study, rats were switched to vehicle injections after 8 weeks on alendronate for another 8 weeks before being challenged with the anabolic stimulus (washout). This recovery period was insufficient for full recovery and the response to SDZ PTS 893 was still greatly reduced after this procedure. Serial pQCT-measurements suggest that part of the interaction happened during the first two weeks of PTH treatment when bone-lining cells are activated by the anabolic drug. In addition bisphosphonate pretreated rats failed to catch up with the vehicle control at all time points suggesting a second level of drug interaction. The failure of the 'washout' period to restore the normal response to PTH is suggestive of a physico-chemical interaction on the level of the matrix embedded bisphosphonate with the overlaying bone lining cells, rather than of direct effects of the drug on osteoblasts or their precursor cells. Overall the data raises the possibility, that bisphosphonate treated patients respond to PTH and SDZ PTS 893 with a delay which could affect the shorter bone mass measurements carried out at 6 months to 1 year. Additionally, bisphosphonate pre-treated rats did not develop the full anabolic response over time. Clinical investigators studying anabolic drugs such as PTH should be aware of potential long-term interactions of bisphosphonates when assessing the outcome of their experiments. However, the beneficial effect of bisphosphonates like alendronate on PTH-induced bone remodeling, as well as its potent action in the protection of bone loss after cessation of anabolic therapy might outweigh the worries about a small delay in the bone response to parathyroid hormone.

Keywords: Bisphosphonates, PTH, Bone

Introduction

Little information is available with regard to drug interactions between standard antiresorptive treatment and parathyroid hormone. Several rat studies suggest that co-administration of calcitonin or estradiol with hPTH(1-34) in rats is well tolerated and that no negative outcome on bone parameters is observed^{1,2,3}. Drug interaction does not necessarily have to evolve on the cellular level. In contrast to calcitonin and HRT, agents like bisphosphonates, which are taken up into the mineral phase of bone, may interact with the lining cell directly (physicochemical interaction).

PTH acts very rapidly to induce bone-lining cells into osteoid synthesizing osteoblasts within 8 to 10 hours after the first administration. At higher doses, the anabolic effect slows down within 4 to 8 weeks. In contrast, the onset of an antiresorptive effect of a low dose bisphosphonate admin-

istered twice weekly is rather slow. For this reason, co-treatment regimens might be unable to pick up a potential interaction occurring early on during treatment. It could thus be important to test the response to anabolic therapy in a sequential protocol in an effort to more closely mimic the clinical situation, which will be encountered at the time of introduction of SDZ PTS 893 or PTH-fragments into the market.

Methods

Skeletally mature retired breeder Wistar rats (16 months of age at baseline, parity 5, weaning recovery 14 weeks) were treated with 28µg/kg s.c. (2 inj/week, s.c. Mon, Thu) or placebo for 16 weeks (Fig. 1). Additional animals were treated for 8 weeks with the same dose of alendronate followed by an 8-week 'washout' period (placebo treatment). All animals were then switched to daily administrations (5 inj/week, s.c., Mon to Fri) of 100µg/kg SDZ PTS 893 for the duration of 12 weeks and the increase in bone mass monitored in the proximal tibial metaphysis by pQCT (XCT-

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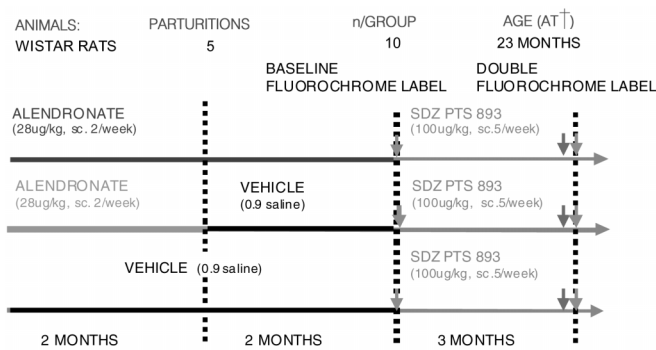


Figure 1: Sixteen month old female rats were pretreated for (a) 16 weeks with alendronate (28µg/kg, s.c., 2x/week), (b) 8 weeks with alendronate and 8 weeks with vehicle (‘washout period’), or (c) 16 weeks with vehicle before they were injected for 12 weeks with the PTH analogue SDZ PTS 893 (100 µg/kg, s.c., 5x/week). They had received a fluorochrome label at the beginning of the anabolic treatment (baseline) and a double label 2 months later.

960, Stratec-Norland) every two weeks. In addition, changes in bone mass in the tibia, femur and lumbar vertebrae were measured at necropsy by DEXA (XR-36, Norland). Biomechanical properties of vertebral body L4, a mid-diaphyseal cortical bone segment from the femur, the femoral neck and the distal femur metaphysis were determined. Rats also received a fluorochrome label at the beginning of the anabolic treatment (baseline) and a double label before necropsy.

Results

Femur bone mass increased by 36% ($p < 0.01$) in animals treated with 100µg/kg SDZ PTS 893 (Fig. 2) compared to the 5% increase observed in the alendronate control group. In rats pretreated for 16 weeks with 28µg/kg s.c. (2 inj/week) of alendronate, the anabolic response was significantly

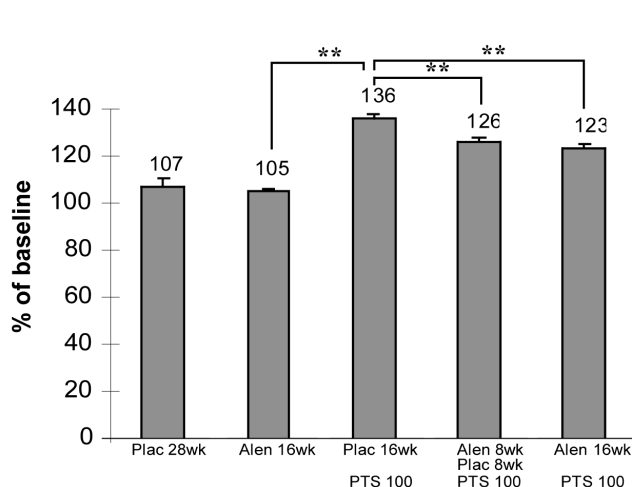


Figure 2: Femur BMC measured by DEXA. Pre-treatment of rats with alendronate (28µg/kg 2inj/wk) significantly blunts the anabolic response to SDZ PTS 893. Withdrawal of alendronate for 8 weeks prior to initiation of anabolic therapy does not restore the ‘normal’ anabolic response.

blunted to 23% ($p < 0.01$). The 8-week ‘washout’ period did not restore the normal anabolic response (26%, $p < 0.01$).

Compression tests carried out in the distal femur metaphysis revealed that SDZ PTS 893 more than doubled (+116%, $p < 0.01$) bone strength compared to a 25% improvement observed in alendronate treated animals (Fig. 3).

Alendronate pre-treatment for 12 weeks significantly reduced the increase in bone strength to 90% ($p < 0.01$) and the 8 week washout-period was unable to restore normal response. (+100%, $p < 0.05$).

Serial pQCT-measurements of cortical thickness revealed that drug interaction occurred at two levels:

1. The reduction in the bone formation response to PTH occurred early on (first two weeks), suggesting that the interaction might take place at the level of the activation of bone lining cells (Fig. 4).
2. Groups of rats pre-treated with alendronate never ‘caught up’ with the vehicle pre-treated group (Fig. 4).

A similar pattern was observed in other pQCT-derived parameters such as cancellous bone mineral density and the cortical bone mineral content.

Histomorphometric analysis of the fluorochrome labels administered 10 and 3 days before necropsy in cross-sections cut from the femur mid-diaphysis confirmed the results from the pQCT. PTH-stimulated osteoblast function was still affected at the end of the study including the washout group, which had received the last bisphosphonate injection more than 19 weeks earlier.

The double labelled surface (Fig. 5) was clearly reduced (ns) in both groups which had received low dose alendronate. Osteoblast performance as indicated by the mineral apposition rate was significantly reduced too (Fig. 6). It is important to note that the site investigated by histomorphometry is a pure modelling site and that PTH induces modeling drifts only. This excludes the possibility, that the

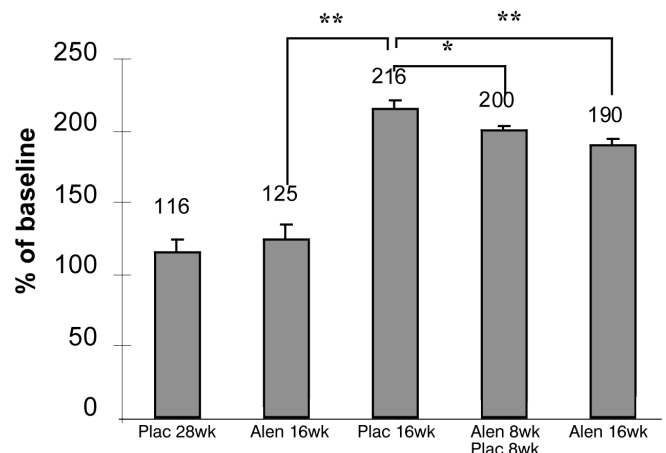


Figure 3: Ultimate strength of the distal femur metaphysis loaded to failure in compression. Pre-treatment of rats with alendronate (28µg/kg 2inj/wk) significantly reduces the improvement in bone strength in response to SDZ PTS 893. Withdrawal of alendronate for 8 weeks prior to initiation of anabolic therapy does not restore the ‘normal’ response.

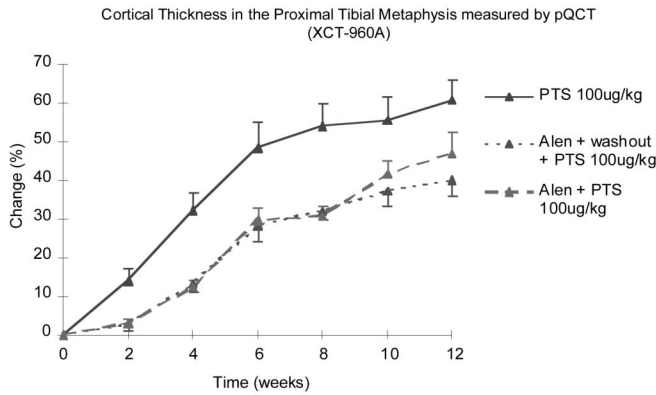


Figure 4: Cortical thickness in the proximal tibial metaphysis measured by pQCT. Pre-treatment of rats with alendronate (28µg/kg 2inj/wk) significantly delayed the onset of the anabolic response to SDZ PTS 893 and the values never caught up during the whole experimental period. Withdrawal of alendronate for 8 weeks prior to initiation of anabolic therapy did not restore the 'normal' response.

observed interaction is mediated by the effect of alendronate on osteoclast mediated bone resorption.

Conclusions

The presence of the bisphosphonate alendronate in the mineralized phase of bone significantly blunts the initial anabolic response to SDZ PTS 893. In part, this blunting is most likely the result of an unknown physicochemical interaction between the matrix embedded bisphosphonate and the first responding bone cells, namely the bone lining cells⁴.

In addition, animals pre-treated with the bisphosphonate never catch up with the group pre-treated with vehicle. Biomechanical tests suggest that the pre-treated animals have less strong bones at the end of the 12-week anabolic treatment even though, according to pQCT measurements, they appear to have reached a plateau.

At present, we can only speculate about the nature of the interaction between the bisphosphonate and the PTH-analogue.

Our experiment tried to distinguish between the two possibilities that alendronate was directly toxic to mature osteoblast populations, lining cells or their precursors, or that the uptake of the drug into the bone matrix was necessary for the adverse effect to occur. This was attempted by comparing an uninterrupted bisphosphonate pre-treatment for 16 weeks with a treatment in which an 8 week course on alendronate was followed by an 8 week placebo treatment (washout). Given the slow rate of release of matrix embedded bisphosphonates into the circulation it could be predicted, that an 8 week washout period would not lead to significant reduction in the matrix concentration of alendronate.

On the other hand, the length of the washout period should have provided sufficient time for recovery of

osteoblast populations from potential direct toxic effects of the compound. The fact that the washout period did not restore the normal pattern of the anabolic response can be interpreted as an indication, that physicochemical interaction of matrix embedded drug, rather than direct inhibitory effects on osteoblast populations may explain our results.

There is now direct evidence in the literature, that bone lining cells are one of the first cells to be activated following PTH-binding to the receptor⁴. Rapid shape change into cuboidal osteoid synthesizing osteoblasts within 6 to 10 hours after drug administration can be observed. Serial pQCT-data showing a roughly 2 week delay in the onset of

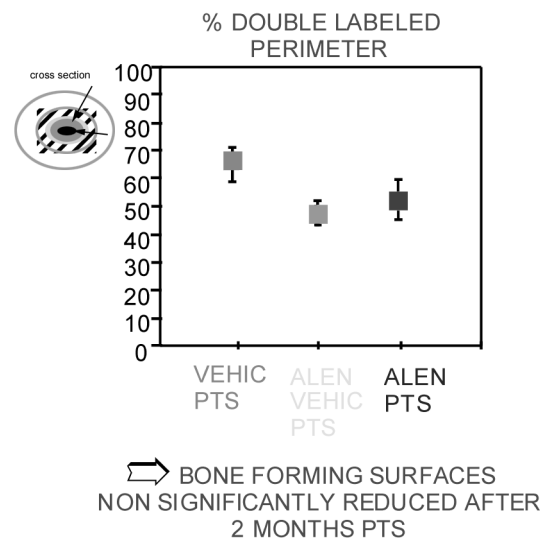


Figure 5: After 2 months of SDZ PTS 893 treatment the amount of bone forming surfaces was non significantly reduced in the two alendronate pre-treated groups compared to the vehicle alone pre-treated group.

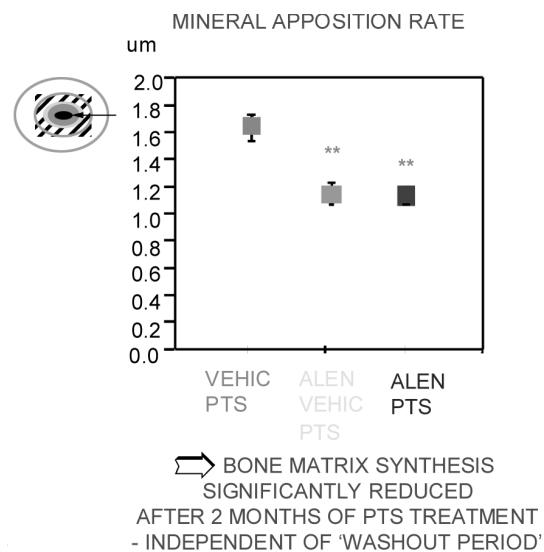


Figure 6: The amount of bone matrix synthesized after the 2 months of the PTH-analogue treatment was significantly smaller in the group which had been pre-treated with alendronate - irrespectively of a 'washout period'.

the anabolic response is also consistent with a direct physico-chemical interaction taking place.

A direct physico-chemical interaction could not account for the decreased mineral apposition detected 2 months after initiation of the treatment. At this time point, bone forming cells were no longer exposed directly to bone matrix containing the bisphosphonate.

The osteocytes on the other hand were not replaced and during alendronate pre-treatment, some were entrapped in matrix into which concomitant uptake of bisphosphonate took place.

It has been speculated, that osteocytes participate in the regulation of the anabolic response to PTH^{5,6}. Alendronate pre-treatment could have rendered osteocytes less sensitive to the action of the bone anabolic drug.

Clinical investigators studying anabolic drugs such as PTH should be aware of potential long-term interactions of bisphosphonates when assessing the outcome of their experiments. Small delays in the onset of the anabolic response may be expected in patients which had previously been treated with bisphosphonates.

It should be noted, that in a remodeling species like the human primate or man, the overall benefit of using a potent anti-remodeling agent might outweigh the disadvantages of a relatively small delay in the anabolic response as well as the small reduction in bone strength.

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