Bone Reports 7 (2017) 70–82

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

Sequential treatment with zoledronic acid followed by teriparatide or vice versa increases bone mineral density and bone strength in ovariectomized rats

T. Shimizu *, T. Tanaka, T. Kobayashi, I. Kudo, M. Nakatsugawa, A. Takakura, R. Takao-Kawabata, T. Ishizuya

Pharmaceuticals Research Center, Asahi Kasei Pharma Corporation, 632-1 Mifuku, Izunokuni, Shizuoka 410-2321, Japan

ARTICLE INFO

Article history:
Received 16 January 2017
Received in revised form 27 May 2017
Accepted 15 June 2017
Available online 19 August 2017

Keywords:
Zoledronic acid
Teriparatide
Sequential treatment
Ovariectomized rat
Bone mineral density
Bone metabolism

ABSTRACT

Bisphosphonates (BPs) and teriparatide (TPTD) are both effective treatments for osteoporosis, but BP treatment prior to daily TPTD treatment has been shown to impair the effect of TPTD in some clinical studies. In contrast, the loss of bone mineral density (BMD) that occurs after withdrawal of TPTD can be prevented by BP treatment. Although various studies have investigated the combination and/or sequential use of BP and TPTD, there have been no clinical studies investigating sequential treatment with zoledronic acid (ZOL) and TPTD (or vice versa). In this study, we evaluated the effects of sequential treatment with TPTD followed by ZOL, and ZOL followed by TPTD, using ovariectomized (OVX) rats.

Two months after OVX, osteopenic rats were treated with ZOL, TPTD, or vehicle for a period of 4 months (first treatment period), and then the treatments were switched and administered for another 4 months (second treatment period).

The group treated with ZOL followed by TPTD showed an immediate increase in BMD of the proximal tibia and greater BMD and bone strength of the lumbar vertebral body, femoral diaphysis, and proximal femur than the group treated with ZOL followed by vehicle. Serum osteocalcin, a marker of bone formation, increased rapidly after switching to TPTD from ZOL.

The group treated with TPTD followed by ZOL did not lose BMD in the proximal tibia after TPTD was stopped, while the group treated with TPTD followed by vehicle did lose BMD. The BMD and bone strength of the lumbar vertebral body, femoral diaphysis, and proximal femur were greater in the group treated with TPTD followed by ZOL than in the group treated with TPTD followed by vehicle. The increase in serum osteocalcin and urinary CTX after withdrawal of TPTD was prevented by the switch from TPTD to ZOL.

In conclusion, our results demonstrate that switching from ZOL to TPTD resulted in a non-attenuated anabolic response in the lumbar spine and femur of OVX rats. In addition, switching from TPTD to ZOL caused BMD to be maintained or further increased. If these results can be reproduced in a clinical setting, the sequential use of ZOL followed by TPTD or vice versa in the treatment of osteoporosis patients would contribute to increases in BMD that, hopefully, would translate into a corresponding decrease in the incidence of vertebral and non-vertebral fractures.

1. Introduction

Bisphosphonates (BPs) and parathyroid hormone (PTH) are antiresorptive and bone anabolic agents, respectively, and both are clinically effective treatments for osteoporosis. However, treatment with a combination of BP and PTH is not necessarily more effective than monotherapy with either one. For example, the increases in bone mineral density (BMD) at the spine and the femoral neck after combined use of daily teriparatide (TPTD; the 1–34 fragment of PTH) and oral alendronate (ALN; a commonly used BP) were smaller than after treatment with TPTD alone in postmenopausal women and in men (Finkelstein et al., 2010; Finkelstein et al., 2003). Moreover, combining PTH (1–84) with daily oral ALN has been reported to inhibit PTH-induced increases in volumetric BMD in women with postmenopausal osteoporosis (Black et al., 2003). Some reports have indicated that BP treatment prior to daily TPTD treatment impaired the effects of TPTD. In postmenopausal women who switched from BP to TPTD treatment, the increases in lumbar spine BMD occurred later, and decreases in proximal femur BMD occurred sooner, in those who were previously treated with daily oral ALN than in those who were previously treated with raloxifene. The effects on BMD of switching to TPTD after

* Corresponding author.
E-mail address: shimizu.tcc@om.asahi-kasei.co.jp (T. Shimizu).

http://dx.doi.org/10.1016/j.bonr.2017.06.001

treatment with risedronate (RIS) were similar to the effects seen in women previously treated with ALN (Cosman, 2014) (Boonen et al., 2008) (Obermayer-Pietsch et al., 2008). Such effects are likely attributable to BP-related inhibition of bone formation, which is coupled to bone resorption.

Zoledronic acid (ZOL) is a BP that is administered once-yearly by intravenous infusion. Among BP agents, ZOL causes the greatest reduction in the vertebral fracture rate in osteoporotic patients (Zhou et al., 2016). As with other BP agents, ZOL causes BMD increases of up to 12% for as long as 6 years after administration (Black et al., 2012). In contrast to the combined effects of ALN and TPTD, the combination of ZOL and daily TPTD has been shown to result in earlier increases in BMD at the vertebral bodies and femoral neck after treatment initiation than monotherapy with TPTD (Cosman et al., 2011). In addition, the combination of TPTD and ZOL has been shown to be more effective than TPTD or ZOL therapy alone in immobilized, osteopenic rats (Vegger et al., 2014). These results suggest that different BP agents, when given in combination with TPTD, may cause different outcomes. While treatment with ZOL followed by TPTD is expected to be effective, there have been no clinical studies that verify the effects of this treatment protocol.

BMD is known to decrease after the termination of TPTD treatment if no subsequent antiresorptive drug therapy is given. Several studies have reported that switching to a BP effectively inhibits such decreases (Kaufman et al., 2005; Kurland et al., 2004; Sugimoto et al., 2013; Prince et al., 2005); however, ZOL was not the BP used in those studies, and no clinical studies have assessed the sequential use of TPTD followed by ZOL. The only report available is a non-clinical study that assessed the efficacy of sequential use of PTH (1–84) followed by ZOL in ovariectomized rats (Rhee et al., 2004); however, in that study the ZOL was administered once per week, and thus the results may not be applicable to the characteristic once-yearly regimen of ZOL used in humans.

In the present study, we used ovariectomized (OVX) rats to assess the effects on bone strength and bone turnover of sequential treatment with ZOL and TPTD using an administration protocol designed to mimic as closely as possible the clinical use of these drugs in humans.

2. Materials and methods

2.1. Animals and experimental design

Two hundred and ten 3-month-old female Sprague-Dawley rats (Charles River, Kanagawa, Japan) were purchased for this study and maintained until 6 months of age to allow them to become skeletally mature. The rats were housed in a dedicated laboratory animal facility with a 12-/12-h light/dark cycle and unrestricted access to tap water and food (CRF-1; standard diet of rats, Oriental Yeast, Tokyo, Japan). All experimental protocols were approved by the Experimental Animal Ethics Committee at Asahi Kasei Pharma Corp. and conducted in accordance with established guidelines concerning the management and handling of experimental animals.

At 6 months of age, the rats were randomly assigned to one of the following body weight-matched groups: baseline (B) group ($n = 6$), OVX group ($n = 172$), or Sham group ($n = 32$). Rats in the OVX and Sham groups underwent bilateral ovariectomy or sham ovariectomy, respectively, while rats in the B group were sacrificed just prior to surgery by exsanguination from the abdominal aorta while under general anesthesia. At 2 months postoperatively ($t = 0$ months), the OVX and Sham rats were randomly assigned to either a main treatment group ($n = 20$ per group) or a satellite group. The groups were matched by proximal tibial BMD and body weight as closely as possible to minimize the differences between them (Fig. 1). Medications were then administered over 8 months divided into two 4-month treatment periods. The effects of sequential ZOL–TPTD and TPTD–ZOL treatments were assessed by treating rats with ZOL during the first 4-month treatment period followed by TPTD during the second 4-month treatment period, or vice versa. The ZOL–TPTD treatment set comprised three groups: the ZOL to TPTD sequential treatment group (Z-T), ZOL to vehicle sequential treatment group (Z-V), and vehicle to TPTD sequential treatment group (V-T). The TPTD–ZOL treatment set also comprised three groups: the TPTD to ZOL sequential treatment group (T-Z), TPTD to vehicle sequential treatment group (T-V), and vehicle to ZOL sequential treatment group (V-Z). Untreated (V-V) OVX and Sham rats were used as controls for both groups (Fig. 1). Satellite groups that were sacrificed before the end of the treatment were set as follows: S0, sham-operated rats sacrificed just prior to the start of treatment ($n = 6$; $t = 0$ months); V0, OVX rats sacrificed just prior to the start of treatment ($n = 8$; $t = 0$ months); S4, sham-operated rats sacrificed after 4 months of treatment with vehicle ($n = 6$; $t = 4$ months); V4, OVX rats sacrificed after 4 months of treatment with vehicle ($n = 8$; $t = 4$ months); Z4, OVX rats sacrificed after 4 months of treatment with ZOL ($n = 8$; $t = 4$ months); T4, OVX operated rats sacrificed after 4 months of treatment with TPTD ($n = 8$; $t = 4$ months). The experimental groups are shown in Fig. 1.

2.2. Experimental treatments and procedures

The S0 and V0 groups were sacrificed just prior to the start of treatment ($t = 0$ months). The other groups were then treated for 4 months (first treatment period) with vehicle (saline 1 mL/kg, single intravenous (IV) injection and saline 0.5 mL/kg/dose, 3 times/week subcutaneous (SC) injection), ZOL (zoledronic acid 100 μg/kg, single IV and saline 0.5 mL/kg/dose, 3 times/week SC), or TPTD (saline 1 mL/kg, single IV and teriparatide 6.0 μg/kg/dose, 3 times/week SC). A total of 100 μg/kg of zoledronic acid (Zometa; Novartis Pharma K.K., Tokyo, Japan) and 6.0 μg/kg of teriparatide (Asahi Kasei Pharma Corp., Tokyo, Japan) were prepared in saline for each injection. At the end of the first treatment period ($t = 4$ months), the S4, V4, Z4, and T4 groups were sacrificed and the main treatment groups were switched to their second period treatments (Fig. 1). After 4 more months (end of the second treatment period; $t = 8$ months), all remaining groups ($n = 14$–20/group) were sacrificed. Although all of the main treatment groups started with 20 animals at the beginning of the first treatment period, the group sizes reduced over time due to euthanasia of some rats for age-related symptoms before the end of the study (Fig. 1). The fourth and fifth lumbar vertebrae (L4 and L5) and the right femur of each rat were harvested at the end of the second treatment period for further analysis.

2.3. Dosage and administration

The typical clinical dosage and administration protocol for ZOL is 5 mg administered by intravenous infusion once yearly. Assuming that the average human body weight is 50 kg, the per body weight dosage is 100 μg/kg. One previous study reported that OVX rats given a single intravenous administration at this dose had BMD and bone strength values at 8 months post-dose that were equivalent to or greater than those of sham-operated animals (Gasser et al., 2008). Thus, we administered ZOL to the OVX rats in the current study at a dose of 100 μg/kg. In extrapolating a once-yearly treatment from humans to rats, consideration must be given to the rate of bone turnover in osteoporotic women and OVX rats. Because bone turnover in OVX rats (Takakura et al., 2016; Ma et al., 1995) is at least three times faster (Takakura et al., 2016) than in women with postmenopausal osteoporosis (Miki et al., 2004; Chavassieux et al., 1997), we selected a dosing frequency of once every 4 months for the treatment of the OVX rats in the present study.

TPTD is available in both a daily and a once-weekly formulation. The approved dosage and administration protocol for the once-weekly formulation of TPTD is 56.5 μg/kg by subcutaneous injection once per week. In past studies, we assessed dosing frequencies and doses of TPTD in rats. The area under the blood concentration-time curve (AUC) in rats given 5.6 μg/kg of TPTD by subcutaneous injection three