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Changes in micro-CT 3D bone parameters reflect effects of a potent cathepsin K inhibitor (SB-553484) on bone resorption and cortical bone formation in ovariectomized mice

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Abstract

Cathepsin K is a cysteine proteinase that is highly expressed by osteoclasts and is being pursued as a potential drug target for the treatment of osteoporosis. We have reported that microcomputed tomography (μCT) analysis of bone microarchitecture may serve as a valuable tool for evaluating both antiresorptive and anabolic agents in ovariectomized (OVX) mice. The purpose of this study was to evaluate the effect of SB-553484, a novel cathepsin K inhibitor (human Ki,app = 0.14 nM, mouse Ki,app = 26 nM), on the OVX mice by μCT bone morphometric analysis. Seven weeks female BALB/c mice were OVX or sham-operated. OVX animals were treated with SB-553484 (30 mg/kg, sc) or Rolipram (10 mg/kg, po), a phosphodiesterase 4 inhibitor used as a positive bone anabolic agent, twice a day for 2 weeks. Both SB-553484 and Rolipram significantly prevented the decrease of trabecular bone volume as well as the deterioration of trabecular architecture in OVX mice. Interestingly, SB-553484 demonstrated a more pronounced effect in improvement of trabecular separation, number and connectivity, and a weaker effect in improvement of trabecular thickness compared to that of Rolipram. These differences indicate that SB-553484 mainly acted as an antiresorptive agent in OVX-induced loss of trabecular bone. On the other hand, SB-553484 significantly increased cortical bone volume and cortical thickness as well as Rolipram in OVX mice indicating an unexpected stimulatory effect of SB-553484 on cortical bone formation. These data suggest that targeting cathepsin K may prove therapeutically beneficial in the treatment of diseases with accelerated bone loss such as postmenopausal osteoporosis not only by inhibiting bone resorption but also by potentially stimulating cortical bone formation.

Keywords: Cathepsin K; Ovariectomized mice; Micro-CT; Bone resorption; Bone formation

Introduction

Osteoporosis is defined as a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture [1]. It is most common in postmenopausal woman, because estrogen deficiency causes an imbalance of osteoclastic bone resorption and osteoblastic bone formation, resulting in deterioration of bone tissue and low bone mass [2,3]. Current therapy for osteoporosis include agents that either inhibit bone resorption (antiresorptive agents) or stimulate bone formation (anabolic agents) thereby leading to a more efficient recovery of bone mass in osteoporosis [4]. As antiresorptive agents, estrogen, raloxifene (selective estrogen-receptor modulator) and bisphosphonates have been used in patients with osteoporosis [3–5]. Parathyroid hormone [PTH (1–34)], an anabolic agent, is also clinically used [4,5]. Rolipram (phosphodiesterase 4 inhibitor) was used in rodent animal models of osteoporosis because it increased bone mass mainly by promoting bone formation in mice and rats [6,7].

Cathepsin K is a cysteine proteinase that is selectively and highly expressed by osteoclasts and has been proposed to play a

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key role in bone resorption [8]. Several groups have developed small molecule inhibitors of cathepsin K that inhibit bone resorption both in vitro and in vivo [9,10]. The inhibition of cathepsin K is expected to suppress osteoclast-mediated bone resorption in post-menopausal osteoporosis. The ovariectomized (OVX) animal model mimics bone loss in the estrogen deficiency condition of postmenopausal woman [11]. In an OVX rat model, changes of two-dimensional (2D) bone parameters by histomorphometric evaluation showed that an inhibitor of cathepsin K significantly prevented the OVX-induced bone loss in trabecular structure and mass [9]. However, it has not been reported as to how cathepsin K inhibitors affect three-dimensional (3D) bone architecture in the OVX model and whether the changes of 3D bone parameters can reflect the effects of cathepsin K inhibitors on bone resorption.

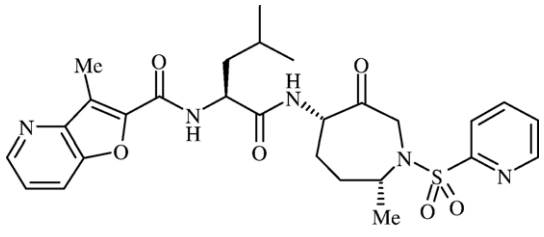
Over the recent years, microcomputerized tomography (μCT) has been used to measure 3D bone structure of small animals because of its relative rapidity compared with conventional histology and its non-invasive feature and its high spatial resolution [12–15]. In our previous study, we have demonstrated that μCT analysis of change patterns of 3D bone parameters may serve as a valuable tool for evaluating both antiresorptive and anabolic agents in OVX mice, and we have also showed a good correlation between the 3D μCT measurements and 2D histomorphometric analysis [16].

The purpose of this study was to evaluate the effect of SB-553484, a novel cathepsin K inhibitor, on bone loss and architecture deterioration induced by ovariectomy using μCT analysis instead of histomorphometry. We described that SB-553484 potently inhibited both human and mouse cathepsin K, and suppressed human osteoclast mediated bone resorption in vitro. In addition, we analyzed the patterns of 3D μCT bone parameter changes in SB-553484-treated animals compared to that of Rolipram-treated one in the OVX mice. Micro-CT bone morphometric analysis of the 3D bone architecture revealed effects of SB-553484 consistent with an inhibition of bone resorption and an unexpected stimulatory effect on cortical bone formation.

Materials and methods

Enzyme assays

Inhibition of human cathepsins B, K, L, S and V and mouse cathepsin K was performed by cleavage of fluorogenic synthetic peptide substrates in the presence of a concentration range of inhibitor, by previously described methods [9,17]. The peptide substrate Cbz-Phe-Arg-AMC was used for cathepsins L, K and V in 100 mM sodium acetate, 5 mM L-cysteine, 5 mM EDTA, 1 mM CHAPS, pH 5.5; Z-Leu-Arg-AMC for cathepsin B in the same assay buffer; whereas Ac-Lys-Gln-Lys-Leu-Arg-AMC was used for cathepsin S in 50 mM MES, 10 mM L-cysteine, 5 mM EDTA, 0.5 mM CHAPS at pH 6.5. Steady state kinetic constants were calculated from the initial rate of product formation.



SB-553484

Fig. 1. Structure of SB-553484.

Table 1
In vitro potency of SB-553484

Enzyme	Ki,app (nM)
Mouse cathepsin K	26.0
Human cathepsin K	0.14
Human cathepsin B	50.0
Human cathepsin L	0.70
Human cathepsin S	0.31
Human cathepsin V	0.31

In situ cytochemical assay

Inhibition of native osteoclast cathepsin K by SB-553484 was tested in an in situ cytochemical assay using sections of human osteoclastoma tissue as described previously [9,18]. Tissue sections were incubated in 100 mM phosphate assay buffer pH 5.3 containing the synthetic substrate Z-Leu-Arg-4MβNA (or Z-Phe-Arg-4MβNA) and SB-553484 (0.03–1 μM) and products detected by azo coupling to Fast Blue BB to generate a red precipitate which was

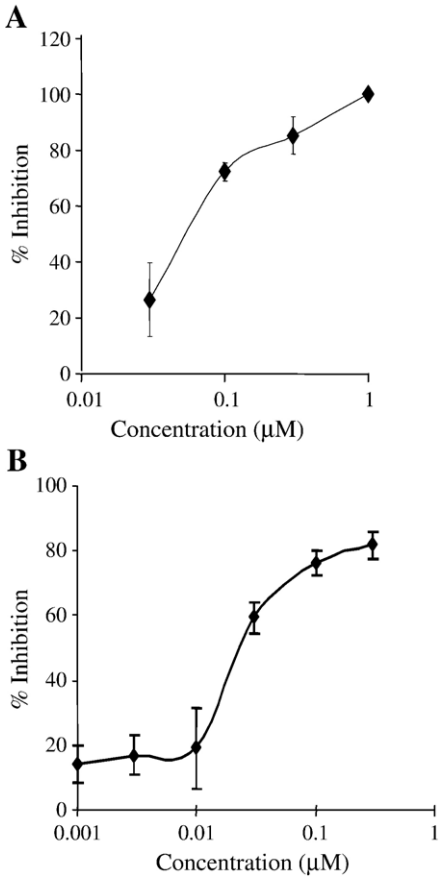


Fig. 2. SB-553484 inhibits human osteoclast cathepsin activity in situ and human osteoclast-mediated bone resorption in vitro. SB-553484 was titrated in the (A) human cathepsin cytochemical assay and (B) human osteoclast resorption assay. The compound was active in both assays with similar potency. Data are reported as IC_{50} values of inhibition of activity in the respective assays.