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Cathepsin K inhibitor causes changes in crystallinity and crystal structure of newly-formed mandibular bone in rats

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Abstract

Cathepsin K inhibitors are new drugs with the potential for the treatment of osteoporosis because they sustain bony remodelling better than bone resorption inhibitors such as bisphosphonates. The treatment of osteoporosis with inhibitors of bony resorption is associated with osteonecrosis of the jaw, as the deterioration in bony quality that they induce is thought to be one of its causes. The quality of bone is delineated by structural and material characteristics (which include the degree and quality of mineralisation, and depends on the content of proteoglycan and the structural integrity of the bony collagen).[1,2] Animal and clinical studies have shown that cathepsin K inhibitors improve the mineral density and structural characteristics of bone, but their effect on the rest remains unknown. We therefore hypothesised that these inhibitors will affect the material characteristics of newly-formed mandibular bone. To verify our hypothesis, we used Raman microspectroscopy to examine such bone in rats that were given a cathepsin K inhibitor, and found unusual crystallinity and an increased substitution of carbonate (CO_3^{2-}) in its crystal structure.

Keywords: Raman spectroscopy; odanacatib; bone quality; mandible; osteonecrosis; ovariectomy

Introduction

The strength of bone depends on its density and quality. Quality depends on its structural and material characteristics, which include its organisation and size, as well as the trabeculae of cancellous bone and the porosity of cortical bone. Material characteristics include the degree and quality of mineralisation, which depends on the content of proteoglycan and the structural integrity of the bony collagen.[1,2]

Cathepsin K, a cysteine protease that is expressed specifically and strongly in osteoclasts, has a central role in the degradation of matrix collagen in bony resorption.[3] Cathepsin K inhibitors are new drugs with the potential for the treatment of osteoporosis, and in a clinical trial, one of these, odanacatib, increased bone mineral density (BMD) and reduced rates of fracture.[4] While inhibitors of bony resorption such as bisphosphonates suppress osteoclastic activity and ultimately induce apoptosis of osteoclasts,[5] cathepsin K inhibitors target only the role of osteoclasts in the degrada-

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tion of the bony matrix.[4] Their mechanism of action should not suppress bony remodelling.

In another clinical trial, odanacatib suppressed markers of osteogenesis only mildly[6] so, unlike existing inhibitors of bony resorption, cathepsin K inhibitors would be expected to treat osteoporosis without destroying the balance between resorption and osteogenesis.[7] However, an osteopetrosis-like phenotype has been found in cathepsin K knockout mice, and pycnodysostosis (which is caused by a mutation in the cathepsin K gene) often results in fibrous dysplasia and stress fractures, even when bone density is good.[8,9] It is therefore important to quantify the effects of cathepsin K inhibitors on the quality of bone.

In the head and neck, inhibitors of bony resorption cause osteonecrosis of the jaw. Currently, the deterioration in bony quality that results from a suppression in remodelling is thought to be a major cause of osteonecrosis,[10,11] and the suppression of bony modelling on the quality of mandibular bone is an area of intense research. To date, however, most studies have focused on the structural characteristics of bone and have not provided sufficient assessment of the material characteristics.[12,13] In this context, Raman microspectroscopy is a powerful tool.[14] It analyses information that is based on molecular vibrations, which allows for non-contact and non-destructive assessment of the molecular structure or crystallinity.

We hypothesised that cathepsin K inhibitors will affect the material characteristics of bone that is in the process of healing after an invasive procedure. We used Raman microspectroscopy to evaluate changes in mineralisation, crystallinity, the content of carbonate and proteoglycan, and the structural integrity of collagen in the mandibles of rats that had been given the cathepsin K inhibitor odanacatib, and compared them with those in rats that had been given no treatment.

Material and methods

Animals

A total of 35 female Wistar rats (oophorectomy n = 23 of which 11 were given odanacatib; sham oophorectomy n = 12) 8 weeks old were obtained from Japan SLC Inc (Hamamatsu, Shizuoka, Japan). They were all operated on four days before arrival in our department. They were kept in a room with controlled temperature and humidity and a 12-hour day and night cycle, and food and water were freely available. All animal studies were approved by the Institutional Animal Care and Use Committee of Okayama University. Experiments conformed to all guidelines and regulations for the protection of the welfare of animals (protocol No. OKU-2016198), and conformed with ARRIVE guidelines. After acclimatisation for 18 days, the rats were assigned to three groups: operation and treatment with odanacatib (n = 11); sham operation only (n = 12); and operation only (n = 12).

Dosing regimens and surgical technique

After acclimatisation for 18 days, the treated rats were given odanacatib orally, 1.8 mg/kg body weight, every day. Two days after the first dose, all rats were operated on under general anaesthesia (medetomidine, midazolam, and butorphanol).[15] An incision was made on the left side of the lateral aspect of the mandibular ramus to the subperiosteum, which was peeled back to the lower and posterior margins of the mandible to show the surgical field. A 1 mm round bur was used to drill a hole bicortically 2 mm from the lower and posterior margins on the left side of the mandible. The wound was closed with sutures. Twenty-eight days postoperatively, all rats were killed under general anaesthesia (medetomidine, midazolam and butorphanol),[15] and the mandibles removed and stored in 70% ethanol at 4 °C for peripheral quantitative computed tomography (pQCT) and Raman analysis.

Analysis of the mandible with pQCT

An area of bone 0.8 mm from the centre of the drilled area on the mandible was scanned with pQCT (Norland/Stratec XCT Research SA+; StratecMedizintechnic GmbH), and BMD (mg/cm^3) was measured in all the rats.

Analysis of the mandible by Raman microspectroscopy

The undecalcified mandibles of all the rats were studied with Raman microspectroscopy (NRS-5000; Jasco). The argon ion laser power (wavelength: 532 nm) for all measurements was 100 mW. Raman spectra were obtained with an acquisition time of 60 seconds twice and summed for each case. Analysis was confined to the Raman scattering region between 800 and 1800 cm^{-1}. Quantitative maps of the newly-formed bone-phase distribution were generated within the sample area (300 μm × 120 μm) using a scanning step of 10 μm. In addition, the outer circumference 100 μm from the outer periphery of the drilled area was examined using an optical electron microscope attached to the Raman microspectroscope. Five spectra/specimen were then randomly obtained around the entire circumference. Peak assignment and interpretation were made according to previously published methods.[16–18] For each of the five spectra, common Raman bone metrics were calculated as follows:

- Mineral:matrix ratio = intensity ratio between v_1PO4(930–980 cm^{-1}) and amide I (1620–1700 cm^{-1}).
- B-type carbonate substitution = intensity ratio between B-type CO_3^{2-} (1050–1115 cm^{-1}) and v_1PO_4 bands.
- Crystallinity = inverse of the full-width at half-height of the v_1PO_4 band.
- Relative proteoglycan content (proteoglycan/organic matrix ratio) = intensity ratio between the

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