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Soluble silica inhibits osteoclast formation and bone resorption in vitro

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ABSTRACT

Several studies have suggested that silicon (Si) may be essential for the normal development of connective tissue and the skeleton. Positive effects of Si from the diet as well as from Si-containing biomaterials, such as bioactive glass 45S5 (BG), have been demonstrated. Studies have reported that Si stimulates osteoblast proliferation and differentiation. However, the effects of Si on osteoclasts have not been directly addressed. The purpose of the present in vitro study was to clarify if Si has regulatory effects on osteoclast formation and bone resorption. The effects of BG, BG dissolution extracts and Si containing cell culture medium were investigated in a mouse calvarial bone resorption assay and osteoclast formation assays (mouse bone marrow cultures and RAW264.7 cell cultures). We conclude from our results that Si causes significant inhibition of osteoclast phenotypic gene expressions, osteoclast formation and bone resorption in vitro. In conclusion, the present study suggests that Si has a dual nature in bone metabolism with stimulatory effects on osteoblasts and inhibitory effects on osteoclasts. This suggested property of Si might be interesting to further explore in future biomaterials for treatments of bone defects in patients.

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1. Introduction

Orthosilicic acid ($Si(OH)_4$) is the simplest soluble form of silica and is utilized by diatoms and sponges in their biosilicification process [1,2]. Studies also support the hypothesis that silicon/soluble silica (Si) may be essential for normal development of connective tissue and the skeleton in vertebras [3–5]. A positive association of dietary Si intake with hip bone mineral density (BMD) in men and premenopausal women was reported in a human cross-sectional cohort study [6]. In the Aberdeen prospective osteoporosis screening study the effects of dietary Si on bone health was confirmed. Interestingly, effects of Si were found to be significantly influenced by the oestrogen status [7]. Already in the early 1970s, the Si deprivation studies by Carlisle [8] and Schwarz and Milne [9] demonstrated the importance of Si on skeletal development and growth in chicks and rats. Further, Si supplementation in ovariectomized (OVX) rats increased BMD and bone formation [10–12], and it was suggested that osteoclast activity was reduced due to a more resilient extracellular matrix caused by Si incorporation [12]. In addition to the positive effects on bone health by dietary Si, it has also been demonstrated that biomaterials containing Si can be used for bone regeneration and repair of bone defects [13,14]. One of the most interesting biomaterials is the Bioglass 45S5 (BG) originally developed by Larry Hench in 1969 [15]. BG ionic dissolution products stimulate proliferation of human osteoblasts and osteogenic markers in vitro and effects may be mediated by Si released from the biomaterials [16–19]. Using two different processing methods, melt-derived and sol-gel-derived routes, a vast number of bioactive glasses with different properties are made. Bioactive glasses vary in size and shape, ranging from nanoparticles to foam scaffolds to middle ear prostheses. The glasses can have different chemical and physical properties which may influence the rate of Si dissolution and the bioactive behavior [14,20]. The majority of studies on Si have focused on bone formation and effects on osteoblasts [18,21–24]. The balance between osteoblast and osteoclasts is important, but little is known about Si effects on the bone resorbing osteoclasts. Osteoclasts are terminally differentiated cells formed by the fusion of mononuclear precursors of hematopoietic origin. The development and activation of osteoclasts require communication with stromal cells/osteoblasts. Osteoclast formation and activation are dependent on receptor activator of nuclear factor κB ligand (RANKL), which is produced by

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osteoblasts/stromal cells [25]. RANKL binds to the RANK receptor on the osteoclast precursors and mature osteoclasts. The RANK–RANKL interaction activates signaling pathways and induces expression of several downstream signaling molecules and transcription factors, including nuclear factor of activated T-cells cytoplasmic 1 (NFATc1). Osteoprotegerin (OPG), also produced by osteoblasts/stromal cells, is a soluble decoy receptor for RANKL and inhibits the RANK–RANKL signaling pathway [26]. Several phenotypic markers for osteoclasts, including tartrate-resistant acid phosphatase (TRAP), Cathepsin K (Cath K) and calcitonin receptor (CTR), are thereafter sequentially expressed during the differentiation process [27–29]. In addition, gap junction cell–cell communication also plays an important role in the regulation of bone cells. Gap junctions are intercellular channels formed by connexin (Cx) integral membrane proteins, of which Cx43 is the most common in bone [30]. Bone resorbing osteoclasts express Cx43 [31,32] and blocking of gap junction cell–cell communication has been shown to inhibit RANKL-mediated osteoclastogenesis [33]. To be able to answer the questions about what effects Si might have on osteoclasts phenotypic markers, cell–cell communication as well as functional aspects (i.e. bone resorption) should be studied. Fluorescent silica-based nanoparticles have been reported to suppress the number of TRAP positive osteoclast-like cells in vitro [34]. It should be noted, as the authors themselves point out, that subtle changes of the silica nanoparticle size, surface and/or shape may led to very different physicochemical properties of the particles in a biological system. It has also been reported that Si could modulate the ratio of osteoclast-regulating cytokines OPG and RANKL in osteoblast-like cells (SaOS-2), and reducing the number of TRAP positive osteoclasts-like cells in a co-culture system [35]. However, direct effects of Si on osteoclast precursors/osteoclasts and osteoclastic bone resorption has not been clarified. The aim for the present in vitro study was to clarify if soluble Si has regulatory effects on osteoclast formation and bone resorption.

2. Materials and methods

2.1. BG and Si

Bioactive glass 45S5 (BG) with particle sizes of 70–700 μm was produced by Dr. Ian Thompson, Kings College, London. The particles were produced using a method previously described in a paper by Koller et al. [36]. We have used particles from the same batch in an earlier study where we characterized the particles with regard to particle size, surface area and porosity [37]. The particles were sterilized by UV illumination for 24 h. In some experiments, a defined amount of BG particles (mg ml^{-1}) was directly used for cell culture experiments. In other experiments, a dissolution extract was prepared from BG under sterile conditions. A defined amount of BG was suspended in 5 ml of α-modification of Minimum Essential Medium (α-MEM; Invitrogen) supplemented with 0.7 mM L-glutamine (Invitrogen), 100 U ml^{-1} bensylpenicillin (Astra Zeneca) and 100 μg ml^{-1} streptomycin (Sigma-Aldrich). BG particles were incubated in α-MEM in plastic tubes (50 × 16 mm, Sarstedt) at 37 °C for 168 h. Thereafter, the non-filtered dissolution extracts were transferred to new tubes. Care was taken not to transfer any granules. Five samples were made for each group for one experiment. All of the dissolution experiments were repeated 2–3 times. The samples were stored at 2–8 °C until analysis by inductive coupled plasma optical emission spectroscopy (ICP-OES) or cell culture experiments. Si-containing medium used in the experiments was prepared by a method previously published by Sripanyakorn et al. [38] with minor modification. A stock solution (350 μg ml^{-1} Si) was prepared by adding 0.1 ml of concentrated sodium silicate (Sigma-Aldrich) to 49.9 ml of α-MEM. The pH was adjusted to 7.0–7.2 using 6 M HCl before 10% (v/v) fetal bovine serum (FBS; Gibco Invitrogen) was added. Final concentrations of 0–200 μg ml^{-1} Si solutions were prepared by diluting the stock solution with α-MEM +10% FBS. Si, Ca and P concentrations were analyzed using ICP-OES (data not shown) to confirm the amount of Si in the medium.

2.2. Inductive coupled plasma optical emission spectroscopy (ICP-OES)

ICP-OES analysis was performed to establish the ion concentrations for Ca, P and Si in the medium using an Optima 2000DV ICP-emission spectrometer and WinLab 32 software (PerkinElmer). Standard solutions were prepared with concentrations of 2000, 100, 50, 10 and 1 ppm Ca, P, Si and Na for the calibration of the instrument. To match the sample matrix, 10% of α-MEM was added to each standard solution.

2.3. Cell cultures

Animal care and experiments were approved by the Animal Ethics Committee at Umeå University (approval no. A27-07 and A40-10) and performed according to the EU Directive 2010/63/EU for animal experiments.

2.3.1. Mouse bone marrow cultures

Mouse bone marrow cells were isolated and cultured as previously described [39]. Cervical dislocation was used to kill 6- to 9-week-old CsA mice. The tibiae and femur were dissected out, and adhering soft tissues was removed. After cutting off the bone ends, the marrow cavity was flushed with cell culture medium (α-MEM supplemented with 10% FBS, 0.7 mM L-glutamine (Invitrogen), 100 U ml^{-1} bensylpenicillin (Astra Zeneca) and 100 μg ml^{-1} streptomycin (Sigma-Aldrich). Next, the marrow cells were collected in tubes (15 ml, Sarstedt), centrifuged at 200*g* for 5 min and resuspended in cell culture medium. Approximately 1×10^6 cells cm^{-2} were cultured in either 35 mm Nunclon culture dishes (for gene expression analysis), 24-well plates (TRAP) or 96-well plates (Neutral Red) (Nunc). The cells were incubated at 37 °C in 5% CO_2 in a humidified tissue culture incubator. After a 24 h attachment period in control medium, the culture medium was replaced, and the cells were cultured in medium with or without recombinant murine RANKL (5 ng ml^{-1}; R&D systems), synthetic bovine parathyroid hormone (10^{-8} M; PTH 1-34; Bachem) or forskolin (10^{-6} M; FSK; Sigma-Aldrich). FSK was dissolved in ethanol, which never exceeded 0.1% v/v in the final solution. BG particles, BG dissolution extract or Si-containing medium was added to marrow cultures as described in figure legends. The culture medium was changed every 2–3 days. After culture for 7–8 days, the cells were either analyzed with quantitative real-time RT-PCR or stained for tartrate-resistant acid phosphatase (TRAP; Sigma-Aldrich) activity. In one experiment, Si-containing medium was present either from days 0–8, 0–2 or 5–8 of culture. The multinucleated TRAP-positive cells formed in the mouse bone marrow cultures expressed markers of differentiated osteoclasts, such as CTR and Cath K. They also had the capacity to excavate resorption lacunae on bone slices [39].

2.3.2. RAW264.7 cell cultures

RAW264.7 cells (American Type Culture Collection) were maintained at 37 °C, 5% CO_2, in α-MEM supplemented with 10% FBS, and antibiotics. The cells were seeded at 5×10^3 cells cm^{-2} in 12, 24 or in 35 mm culture dishes (Nunc) for quantitative real-time RT-PCR analysis or TRAP assays or at 10^4 cells cm^{-2} in 96-well plates (Nunc) for Neutral red uptake assays. After a 24 h attachment period in control medium, the cells were further cultured in medium with or without RANKL, BG particles, BG dissolution extract or Si-containing medium. The culture medium was changed after 2–3 days, and analyses were performed after 3–5 days.

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