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Silicon and boron differ in their localization and loading in bone ☆

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ABSTRACT

Silicon and boron share many similarities, both chemically and biochemically, including having similar effects on bone, although their mechanisms of action are not known. Here we compared the loading of silicon and boron into bone, their localization and how they are influenced by age (growth & development), to obtain further clues as to the biological effects of these elements and, especially, to see if they behave the same or not. Bone samples were obtained from two different studies where female Sprague Dawley rats had been maintained on a normal maintenance diet for up to 43 weeks. Total bone elemental levels were determined by ICP-OES following microwave assisted acid digestion. Silicon and boron levels in the decalcified bones (i.e. the collagen fraction) were also investigated. Silicon and boron showed marked differences in loading and in their localization in bone. Highest silicon and lowest boron concentrations were found in the under-mineralized bone of younger rats and lowest silicon and highest boron concentrations were found in the fully mineralized bone of the adult rat. Overall, however total bone silicon content increased with age, as did boron content, the latter mirroring the increase in calcium (mineral) content of bone. However, whereas silicon showed equal distribution in the collagen and mineral fractions of bone, boron was exclusively localized in the mineral fraction. These findings confirm the reported association between silicon and collagen, especially at the early stages of bone mineralization, and show that boron is associated with the bone mineral but not connective tissues. These data suggest that silicon and boron have different biological roles and that one is unlikely, therefore, to substitute for the other, or at least boron would not substitute for Si in the connective tissues. Finally, we noted that silicon levels in the mineral fraction varied greatly between the two studies, suggesting that one or more nutritional factor(s) may influence the loading of Si into the mineral fraction of bone. This and the nature of the interaction between Si and collagen deserve further attention.

1. Introduction

Silicon (Si) and boron (B) share many similarities, both chemically and biochemically. Both are non-metals and semi-conductors that can exist in amorphous and crystalline states and form organo compounds containing Si–O (Cox, 2009; West and Barton, 1980) or B–O bonds (Hunt, 2012; Devirian and Volpe, 2003). Both also form weak acids upon dissolution in water that are fully pronated (pKa_1 9.6 and 9.3, respectively) and, therefore, neutrally charged at physiological pHs. Silicon and B are quasi-essential/essential for some plants and algae (Camacho-Cristobal and Gonzalez-Fontes, 2008; Ju et al., 2011; Epstein, 1999; Brzezinski et al., 1990; Hildebrand et al., 1997). Both elements are deposited in the cell walls of plants where they are reported to have a structural role, affecting cell wall properties such as porosity and preventing pathogen attack (Camacho-Cristobal and Gonzalez-Fontes, 2008; Epstein, 1999; Fauteux et al., 2006). Specifically, B binds and stabilizes cell wall components containing cis-diol groups such as pectins and the apiose residues of rhamnogalacturonan II (Camacho-Cristobal and Gonzalez-Fontes, 2008). Kinrade et al., and Wang et al., have shown similar binding by Si (Jugdaohsingh et al., 2008a; Wang, 2004). The lack of either Si or B causes cell cycle arrest in diatoms, thus affecting their normal growth (Hunt, 2012; Brzezinski et al., 1990). Boron depletion has also been shown to prevent completion of the life cycle (embryo survival) in zebrafish and frogs, as well as preventing limb development in frogs (Hunt, 2012; Nielsen, 2008). Similar studies with Si have not been reported.

In higher animals, dietary Si or B deprivation is reported to impair normal growth and bone development (Hunt, 2012; Nielsen, 2008; Carlisle, 1972; Schwarz and Milne, 1972). The lack of Si or B is also reported to affect growth plate maturation and to impair growth plate closure (Hunt, 2012; Jugdaohsingh et al., 2008b). Higher intakes of Si,

Abbreviations: B, boron; EDTA, ethylenediaminetetraacetic acid; ICP-OES, inductively coupled plasma-optical emission spectroscopy; Si, silicon; SGIF, simulated gastrointestinal fluid; UHP, ultra-high purity

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or Si supplementation, are associated with higher bone mineral density (BMD (Jugdaohsingh et al., 2004; Macdonald et al., 2012; Eisinger and Clairet, 1993)) and better trabecular micro-architecture (Jugdaohsingh et al., unpublished data & ref. Nielsen and Stoecker, 2004). Boron supplementation similarly increases BMD, trabecular micro-architecture and bone strength (Hunt, 2012; Nielsen, 2008; Price et al., 2013; Nielsen and Stoecker, 2009).

The exact role of Si or B in bone health is however not known. There is a suggestion that B may increase the efficacy or utilization of vitamin D (Hunt, 2012; Devirian and Volpe, 2003; Nielsen, 2008). Indeed, it has been reported that B can alleviate marginal vitamin D deficiency (Hunt, 2012). Boron has also been reported to increase the levels of steroid hormones (e.g. estrogen and testosterone) in serum by influencing their metabolism (Devirian and Volpe, 2003; Nielsen, 2008). Silicon deficiency also affects the normal development of other connective tissues, not just bone (Carlisle, 1972; Schwarz and Milne, 1972), and thus it has been suggested that Si may be involved in the synthesis and/or stabilization of the collagen matrix.

Thus while the above findings suggest that Si and B share similar effects on bone, they may bring about these effects through different pathways/routes. Here we show that dietary Si and B show marked differences in bone loading with age and also differences in bone localization, providing further clues as to the biological effects of these non-metals.

2. Materials and methods

2.1. Bone samples

Bone samples were obtained from two studies utilizing female Sprague Dawley rats. In both studies, rats were maintained under standard conditions; i.e. in plastic cages with stainless steel cover at 22 °C, with 12/12 h light/dark cycle and with ad libitum access to a standard maintenance diet.

2.1.1. Study

Paired adult humerus bones (n = 5 pairs) were obtained from a reference group of adult (19 week old) Sprague Dawley rats from a previous study (Jugdaohsingh et al., 2008b). In brief, female Sprague Dawley rats (B&K Universal Ltd.; Hull, UK) were maintained, from three weeks of age, on B&K Rat and Mouse Standard Diet (B&K Universal Ltd.) and tap water for 26 weeks and thereafter killed (by a Schedule 1 method of euthanasia approved by the Home Office Animals Scientific Procedures Act 1986) following an overnight fast. Bone and other tissues were collected (Jugdaohsingh et al., 2008b). Paired humerus bones were cleaned of excess flesh, tendons and ligaments and stored frozen at −80 °C until analysis.

Prior analysis had shown that the B&K Rat and Mouse Standard Diet contained, on average, 322 μg Si/g feed and 7.4 μg B/g feed (Table 1 & ref. Jugdaohsingh et al., 2008b). The drinking water contained 5 mg Si/L, while B was undetected.

Table 1
Silicon, boron and calcium contents of the maintenance diets used in Study 1 and Study 2 analyzed directly by the authors.[a]

	B&K Rat and Mouse Standard Diet (Study 1)	SDS RM1 Expanded Diet (Study 2)
Si (μg/g)[b]	322 (47)	628 (66)
B (μg/g)	7.42 (0.62)	9.12 (0.50)
Ca (mg/g)	8.85 (1.48)	11.76 (1.08)[c]

[a] Nutrient contents of the diets, supplied by the manufacturers, are listed in Supplementary Table 1.
[b] Bioavailability of Si from the two diets were similar: 37% and 33% for the B&K and SDS RM1 diets, respectively (see Supplementary data).
[c] Note, this is higher than the 7.3 mg/g given by the feed company (Supplementary Table 1).

2.1.2. Study 2

Paired tibia bones were collected from female Sprague Dawley rats of six different ages (3, 5, 8, 12, 26 and 43 weeks), at the National Heart and Lung Institute, Royal Brompton Hospital, London, UK (Jugdaohsingh et al., unpublished data). These rats were 'surplus to requirement' and had been maintained on a standard maintenance diet, namely SDS RM1 Expanded Diet (Special Diets Service, UK), and drinking water from three weeks of age. Following overnight fast, rats were sacrificed by Schedule 1 method and bones and tissues were collected. Paired tibia bones were cleaned of excess flesh, tendons and ligaments and stored at −80 °C until analysis.

Samples of the maintenance diet and drinking water were analyzed for Si, B and Ca (Table 1). The drinking water contained 3.9 mg/L Si and 0.051 mg/L B.

2.2. Materials

Ethylenediaminetetraacetic acid (EDTA, disodium salt dihydrate, 99% purity), high purity nitric acid (69% p.a. plus), 1 M hydrochloric acid, sodium hydrogen carbonate ($NaHCO_3$) and pepsin from porcine gastric mucosa were from Sigma Aldrich Chemical Co. (Gillingham, UK). Sodium chloride was from Fluka Analytical (UK). Dialysis membranes (12.5–14 kDa) were from Spectrum Lab Inc. (UK). ICP standard solutions, 1000 mg analyte/L were from Merck Ltd. (Poole, UK) or Sigma-Aldrich Chemical Co. (Gillingham, UK). Polypropylene sample collection tubes (13 mL & 50 mL) were from Sarstedt Ltd. (Leicester, UK). Ultra high purity (UHP) water was 18 MΩ/cm, from a Branstead Nano-Pure water purifier (Thermo Scientific; Ohio, USA). A 100 mg/L multi-element standard was prepared by pooling 1 mL aliquots of the 1000 mg/L ICP standard solution of the elements of interest.

2.3. Decalcification of bone samples

One of each of the pairs of humerus and tibia bones collected in Studies 1 and 2, respectively, were decalcified to determine what proportions of the total bone Si and B were associated with the collagen component of bone.

Whole right humeri (n = 5, from Study 1) were placed in individual dialysis bags with 5 mL of a saturated EDTA solution (37 g in 500 mL UHP water) inside the bag and 40 mL outside the bag in a 50 mL polypropylene tube. Each bone sample was individually decalcified for 4 weeks, at 4 °C. The EDTA solution, inside and outside, was changed every 2/3 days. The decalcified bones were then incubated in UHP water at 4 °C, for five days and then stored at −80 °C until analysis.

Whole left tibia (n = 3 per age group, except for the 3-week old rats from Study 2 due to insufficient mass) were similarly decalcified as described above, but over a shorter (2-week) period, as this was found to be more than adequate to remove the mineral phase (Supplementary Fig. 1). The decalcified bones were similarly incubated with UHP water and then stored at −80 °C until analysis.

2.4. Total elemental analysis

Total elemental analyses were carried out by inductively coupled plasma optical emission spectrometry (ICP-OES: Jobin Yvon 2000-2, Instrument SA, Longjumeau, France), equipped with a concentric nebulizer and cyclonic spray chamber. Sample flow rate was 1 mL/min. Peak profiles were used as previously described (Burden et al., 1995; Sripanyakorn et al., 2009), with a window size of 0.08 nm (0.04 nm either side of the peak) with 21 increments per profile and an integration time of 0.5 s per increment.

Analytical lines were: 251.611 nm (Si), 208.96 nm (B), 213.618 nm (phosphorus, P), 315.887 nm (calcium, Ca), 279.806 nm (magnesium, Mg), 766.490 nm (potassium, K), 257.610 nm (zinc, Zn), 259.940 nm (manganese, Mn) and 324.754 nm (copper, Cu). All samples were analyzed in a blinded fashion.