Long-Term Evaluation of Blood Silicon and Ostecalcin in Operatively Treated Patients With Benign Bone Tumors Using Bioactive Glass and Autogenous Bone

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Abstract: In a study on 25 patients with verified benign bone tumors, bioactive glass (BG) and autogenous bone (AB) were used as bone-graft substitutes. The patients were randomized into two groups according to the filling material. Blood samples were taken both preoperatively, at 2 weeks, and 3, 8, 12, 24, and 36 months postoperatively, for evaluation of silicon concentration in blood. In the determination, direct current plasma atomic emission spectroscopy was used. No significant difference in blood silicon concentration between the BG group or the AB group could statistically be observed ($p = 0.5400$), and neither did the size of the bone tumor ($p = 0.4259$) nor the follow-up time affect the results ($p = 0.2094$). Concentration of osteocalcin in blood was significantly higher for large cysts ($p < 0.0001$). The filler material (BG or AB) did not affect the osteocalcin concentration level in blood. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 87B: 73–76, 2008

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INTRODUCTION

Silica, referring to silicon bonded to oxygen, is the most abundant mineral in the earth's crust.[1] Soluble silica (silicic acid) is ubiquitous in the diet and in natural waters. In the human body, silicon is present as a trace element and found in very small amounts.[2]

Bioactive glasses used as biomaterials also contain silicon. As the glass is implanted in the body a chemical reaction starts at the surface of the glass. The reaction layer consists of a silica-rich layer, and on top of this layer, a hydroxyapatite layer is formed.[3,4] The aim of this study was to evaluate the effect of implanted bioactive glass (BG) granules on blood silicon and osteocalcin concentration in bone tumor surgery. The study was conducted at the Department of Orthopaedics and Traumatology of the University Hospital of Turku, Finland. Local ethics committee approval and patient-informed consent was obtained for this clinical study.

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MATERIALS AND METHODS

Twenty-five patients with radiologically diagnosed benign bone tumors participated in this study. The tumors were located in the proximal humerous (6), distal tibia (7), digits of the hand (9), naviculare (1), patella (1), and talus (1).

The bone tumors were preoperatively diagnosed by X-ray and MRI. In the operation, a cortical fenestration was made for biopsy of the tumor. The type of tumor was classified by histopathological examination as follows: aneurysmal bone cyst (3), enchondroma (8), nonossifying fibroma (5), cysta simplex (7), cysta epidermalis (1), and desmoplastic fibroma (1). The cystic lesion was evacuated by curettage. The walls of the cavity were carefully drilled to refresh the bone. The cavity was filled either with bioactive glass (BG), S53P4 (composed of 53% SiO_2, 23% Na_2O, 20% CaO, and 4% P_2O_5)[5] of a granule size of 630–3150 μm or autogenous bone (AB) taken from the iliac crest, according to a preoperative randomization of the patients. The bioactive glass, S53P4, (BonAlive®, Vivoxid, Finland) gained European approval for its orthopedic use as a bone substitute in 2006. BG was used in 14 patients (BG group) and AB in 11 patients (AB group). Of the bone tumors, 64.3% in the BG group and 63.6% in the AB group were

TABLE I. Mean Blood Silicon Concentration (μg/g) for Large and Small Bone Tumors

Time	Size of Tumor	AB, Mean/SD (μg/g)	BG, Mean/SD (μg/g)
Preoperative	Large	64.90/12.49	57.90/35.92
	Small	66.91/9.33	61.52/16.49
2 weeks	Large	63.88/27.37	62.62/16.24
	Small	55.11/7.04	59.24/8.68
3 months	Large	71.18/16.50	71.11/17.83
	Small	47.19/5.52	62.11/10.14
8 months	Large	65.90/14.34	67.99/13.08
	Small	65.02/13.01	63.79/16.03
12 months	Large	59.03/35.01	60.35/48.94
	Small	67.76/25.31	83.06/–
18 months	Large	–	9.90/–
	Small	75.04/11.62	–
24 months	Large	52.92/35.90	42.53/36.13
	Small	38.10/31.07	84.83/15.61
36 months	Large	10.47/4.48	21.28/21.02
	Small	65.12/4.45	71.38/1.83

AB, autogenous bone; BG, bioactive glass.

classified as large (mean 28.8/23.4 cm^3) and 35.7%/36.4% as small (mean 1.1/2.3 cm^3). Routine antibiotics and antithromboembolic prophylaxis were used in both groups, as well as antiinflammatory drugs for pain relief.

Blood Analyses

Blood samples were taken preoperatively, at 2 weeks, and 3, 8, 12, 24, and 36 months postoperatively.

Determinations of total silicon concentration in blood was performed by direct current plasma atomic emission spectroscopy (DCP-AES) using a Spectraspan IIIB (Spectra Metrics). Sample concentrations were reported in micrograms of silicon per gram of blood. Each value was based on three measurements of a given sample.

Concentration of osteocalcin and blood parameters (B-Hb, B-Hkr, B-eryt, B-MCV, fB-leuk, B-tromb, P-CRP, P-K, P-Na, fP-Crea, fP-Ca, and P-AFOS) were also measured according to standard methods.

STATISTICAL METHODS

All primary and secondary outcome statistical analyses were performed by an independent source (Medikalla Oy, Medfiles, Turku, Finland). The intent-to-treat populations, which included all randomized patients, were used in all tables and analyses. Descriptive statistics were calculated for all variables. Categorical variables were presented in frequencies tables (PROG FREQ in SAS®) (number of cases and percentages) by treatment. The numerical variables were tabulated by treatment of PROG UNIVARIATE in SAS. Concentration of silicon in blood and concentration of osteocalcin were analyzed with analysis of variance for repeated measures when treatment, size of the tumor, time and treatment × time interaction were in the model (PROG MIXED in SAS).

All statistical evaluations utilized SAS procedures in SAS system for Windows (Version 8.2).

RESULTS

The mean concentrations of silicon in blood for the BG and AB groups are shown in Table I. No difference between the BG group and AB group in the concentration of silicon in blood could statistically be observed ($p = 0.5400$) at any time ($p = 0.2094$), nor did the size of the bone tumor affect the concentration of silicon in blood ($p = 0.4259$).

However, the only change, a nonsignificant numerical trend to elevated Si values, was observed when large and small tumors were compared at 3 months.

For osteocalcin, a correlation between the size of the cysts and the concentration of osteocalcin in blood was observable (Figure 1). The concentration of osteocalcin was significantly higher for large cysts ($p < 0.0001$). However, no other correlation between implantation of BG and AB could be found.

DISCUSSION

Silicon is mainly absorbed from the gastrointestinal tract as orthosilicic acid ($Si(OH)_4$). Depending on the diet, it provides 10–50 mg Si/day. Intake of food alone has been shown to result in a rise of the level of Si in serum. Silicon has, however, also been shown to be readily excreted in urine, with subsequent return of serum silicon concentrations to baseline.[6]

Soluble silicon unlike crystalline silica has no associated toxicity, and many positive effects of silicon have been demonstrated. It has been postulated to be a physiologically important element, as silicon deprivation has shown to decrease concentrations of calcium, copper, potassium, and zinc in femoral bone in rats.[7]

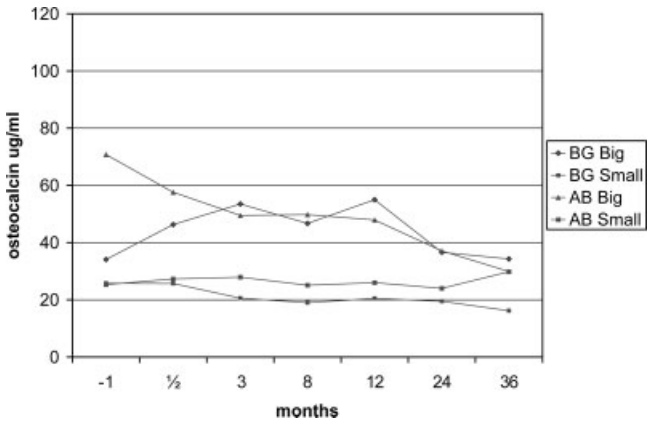

Figure 1. Osteocalcin concentration in blood. The size of the filled cavity influences the osteocalcin level regardless of filler material.

The antagonistic interactions between silicon and aluminium in living organisms have also been debated.[8] Dissolved silicon has been shown to be an important factor in limiting the absorption of dietary aluminium.[9] Jugdaohsing et al. have shown that inhibition of alumina is depending on the chemical structure of silica, and differentiate between monomeric silica ($Si(OH)_4$) and oligomeric silica. Strong interactions have been observed *in vitro* between oligomeric silica and aluminium in the human gastrointestinal tract, with subsequent reduction in the absorption of aluminium.[10] Interactions between silicon and arginine have been postulated to affect immune function, as inadequate dietary silicon impairs splenic lymphocyte proliferation.[11]

The amount of silicon has been measured by DCP-AES in tissues in nonaugmented cadaveric patients as well as in patients augmented for silicon in breast implants. The reported values in nonaugmented patients were 0.2–45 μg/g and for the augmented patients 200–1600 μg/g.[1]

In our study, the blood values ranged from 6.42 to 146.39 μg/g, remaining at a low level. This may depend on several reasons. The intercellular human body fluid space volume is large in relation to the amount of bioactive glass. The degradation rate for bioactive glass is slow, especially for glass granules of 3150-μm size.

Bioactive glass is a bone substitute with a chemically bone bonding ability. The bonding can be presented as a complex series of reactions in the glass and at the glass surface. When the glass is implanted in the bone, a first rapid reaction starts at the glass surface, with an exchange of Na^+ and K^+ ions, with H^+ or H_3O^+ from the surrounding solution. These reactions produce an alkaline environment with subsequent breaking of —Si—O—Si— bonds in the glass. This dissolution occurs locally and results in the formation of silanol groups (SiOH) at the glass interface. A repolymerization then takes place when hydrated silica groups repolymerize with silanol, and a Si-rich layer is formed on the glass surface. These reactions occur minutes after the implantation. On top of this layer, a $CaO–P_2O_5$ stabilizing layer is formed as a result of Ca^{2+} and PO_4^{3-} migration from the glass to the surface.

Our results show that bioactive glass implanted in bone cysts does not significantly affect silicon concentration in blood. This was demonstrated for each patient as a long-time, follow-up study. The amount of implanted material according to the size of the cysts does not affect the silicon concentration in blood.

Serum creatinine, which shows the efficacy of renal function, was also measured for each patient. During the study, the creatinine values stayed at a normal level. Whether there exist a serum or plasma creatinine value area, it is not known which area is unsafe for bioactive glass implantation.

In an *in vitro* model for frontal sinus obliteration with bioactive glass S53P4, it has been shown that when glass S53P4 is placed in a simulated body fluid, a cumulative loss of silicon and phosphate can be observed. The amount of dissolved silicon and phosphate during the 6-month's follow-up study was small, and therefore, glass S53P4 was considered to be a stable, durable, and safe material for massive filling of frontal sinuses.[12] This is in accordance with our study, as no difference in silicon blood concentration between the two groups could be observed. The simulated body fluid is, however, thought to simulate the clinical situation, and therefore, it can be assumed that locally around the implanted glass in bone a rise in silicon concentration is possible. How does this affect bone formation or the surrounding tissue?

In 1970, Carlisle postulated that silicon is associated with calcium in the mineralization process of bone.[13] Two years later, Carlisle and coworkers showed that silicon in chicks exerted growth, and that the leg of the deficient birds had shorter and thinner bones, as well as abnormally shaped bones. This was the first time that it was shown that silicon itself could be considered as a participant in normal bone metabolism.

Whether silicon has a direct effect on bone mineralization or not is unclear. Silicon deprivation decreases collagen formation in bone in rats. It has been suggested that silicon is an important nutrient in wound healing as well as in bone formation.[14,15] Orthosilicic acid ($Si(OH)_4$) at physiological concentrations has been shown to stimulate type 1 collagen synthesis in human osteoblast-like cells and skin fibroblasts, and enhance osteoblastic differentiation.[16] Silicon is also known to bind to glycosaminoglycans and has shown to participate in the formation of crosslinks between collagen and proteoglycans, resulting in stabilization of bone matrix.[17] Dissolution studies on a Si-rich nanocomposite *in vitro* also suggest a role of dissolved silicon in stimulating the differentiation and mineralization of osteoblast precursor cells.[18] Depression of silicon has, however, not been shown to affect bone calcium content or bone-breaking characteristics, indicating that silicon does not have a major effect on bone-crystal formation once the mineralization process has been initiated.[19]

According to these findings, silicon from the bioactive glass may have a positive effect on bone formation indirectly, though the effect of silicon on bone matrix synthesis as type 1 collagen has been shown to enhance the expression of the differentiated osteoblasts by increase in mineralization.[16]

Serum osteocalcin has been found to correlate with bone formation. Alkaline phosphatase and osteocalcin activity has been shown to increase during cell treatment with silicon *in vitro*.[16] We also measured the osteocalcin concentration in blood, but no correlation with implantation of bioactive glass or AB could be found. However, a correlation between the size of the cysts and the concentration of osteocalcin was observable, as the values were significantly higher for large cysts ($p < 0.0001$).

The osteocalcin values for the large bone tumors stayed at a higher level from 3 to 12 months, referring to an increased metabolic activity that has been observed in pre-

vious studies. Increased metabolic activity after implantation of BG in bone has been seen at 3 months by MRI and single photon emission computed tomography.[20]

The participation of silicon in the bone-mineralization process and metabolism in the human body is not fully understood. According to the literature it, however, seems like silicon has a positive effect on bone formation. Therefore, concerning bioactive glasses, it can also be assumed that silicon itself can locally promote the bone-forming process and mineralization. During the follow-up period, no elevation of silicon concentration in blood was observable. Bioactive glass can, therefore, also be considered as a body-biotolerant material.

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