



CLINICAL STUDIES

The concentration of manganese, iron, and strontium in hip joint bone obtained from patients undergoing hip replacement surgery



Halina Budis^{a,b}, Elzbieta Kalisinska^a, Natalia Lanocha^a, Danuta Kosik-Bogacka^{a,*}, Sebastian Sokolowski^c, Konrad Dobiecki^c, Lukasz Kolodziej^c, Andrzej Bohatyrewicz^c

^a Department of Biology and Medical Parasitology, Pomeranian Medical University, Powstancow Wielkopolskich 72, 70-111 Szczecin, Poland

^b Department of Health Education, University of Szczecin, Piastow 40B, 71-065 Szczecin, Poland

^c Chair and Clinics of Orthopedics and Traumatology, Pomeranian Medical University, Unii Lubelskiej 1, 71-252 Szczecin, Poland

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ABSTRACT

The aim of this study was to determine the concentrations of manganese (Mn), iron (Fe) and strontium (Sr) in the cartilage with adjacent compact bone and spongy bone collected from patients after total hip replacement surgery. In addition, we examined relations between the concentrations of the metals in the bone and selected environmental factors. The concentration of Fe was the highest while Mn concentration was the lowest. The concentrations of Fe in the spongy bone in patients from larger cities were higher than in those living in smaller towns and villages. Significant correlations were found between Fe and Mn concentrations in the cartilage with adjacent compact bone and in the spongy bone, and between Mn and Sr in the spongy bone. In general, Mn, Fe and Sr concentrations in the bones of patients from NW Poland were lower than in other Polish regions and Europe, especially in industrialized countries. In conclusion, it seems that in addition to routine monitoring of the abiotic environment, it is essential to monitor concentrations of heavy metals having a long-term impact in humans.

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Introduction

Iron (Fe) has been shown to be involved in the synthesis of collagen and the conversion of 25-hydroxy vitamin D into an active form [1–3]. The role of Fe in cartilage and bone formation and its impact on the occurrence of bone disease in humans is not well-known. A few works have shown that Fe is necessary for the proper functioning of osteoblasts, responsible for osteogenic processes [4]. Its effect on bone mass density (BMD) has been shown in healthy subjects [5,6]. A few studies have reported the effect of excess Fe on osteoblast dysfunction and metabolic bone disorders, including osteopenia, osteoporosis and osteomalacia in humans [7,8].

Manganese (Mn) is a component of various enzymes involved in the metabolism of carbohydrates and fats, essential for the functioning of the immune system and the formation of cartilage and bone [9]. In particular, Mn is involved in the process of ossification and mucopolysaccharide synthesis in cartilage [10,11]. Manganese deficiency may cause growth retardation, skeletal deformities, problems with glucose tolerance [9] and seizures [12]. Bone disorders caused by Mn deficiency are the direct result of enzymatic defects in the synthesis of glycosaminoglycans. Excess Mn may cause disturbances in the metabolism of other elements, such as

Fe, resulting in the inhibition of hemoglobin formation [13]. Manganese introduced in excessive quantities has a neurotoxic and osteotoxic effect, and disrupts the physiology of many tissues and organs [14,15].

Strontium (Sr) belongs to the group of alkali metals, with properties similar to calcium. Almost 99% of absorbed Sr is deposited in bones and teeth. The effect of Sr on bones is not completely understood. It is an antagonist of calcium-sensing receptor (CaR), so can be used as a substitute for calcium supplementation in disorders of the skeletal system. Doses from 200 to 1800 mg/kg/d of Sr reduce resorption and stimulate bone formation, causing an increase in their weight [16]. The presence of Sr is closely linked with calcium balance in osteocytes – it enhances differentiation of osteoblasts, while inhibits the differentiation of osteoclasts and their osteoclastic functions [17]. Strontium also increases the synthesis of collagen and non-collagenous proteins [18]. Strontium ranelate, widely used in recent years, promotes remineralization of bone, especially in postmenopausal women [19,20]. Strontium supplementation stimulated growth and bone mineralization [21,22]. An excess of Sr disrupts calcium and phosphate metabolism, which may result in abnormal bone mineralization and, consequently, lead to bone deformities [22,23].

In our previous studies we determined the concentrations of copper, zinc, lead, cadmium and mercury in the hip joint bones of surgery patients [24]. This study was to determine the concentrations of other elements (Mn, Fe and Sr) in the cartilage,

* Corresponding author. Tel.: +48 91 4661672; fax: +48 91 4661671.

E-mail address: kodan@pum.edu.pl (D. Kosik-Bogacka).

compact bone and spongy bone of patients taken following total hip replacement surgery, and to examine the relations between the concentrations of metals in the investigated bone material and selected environmental factors.

Materials and methods

Materials

The objects of the studies were elements of hip joints from 37 patients living in the West Pomerania Province, NW Poland, who had undergone total hip joint replacement surgery. The study group comprised men aged between 53 and 78 years (mean 62.6 years) and women aged between 32 and 82 years (mean 69.9 years). The exact characteristics of the study group are shown in a previous work on the same population [24]. The studies were approved by the Bioethics Committee of the Pomeranian Medical University in Szczecin (KB-0080/114/09). From each patient three types of material were obtained from the femoral head: cartilage, compact bone and spongy bone. In some samples articular cartilage was pathologically altered. There was also a significant loss of articular surfaces, and due to the advanced coxarthrosis, cartilage was scarce or even non-existent. As a result of hyperemia, the subcartilaginous layer (compact bone) was subject to softening, disintegration and focal destruction. There was a strong variation of compact bone thickness – from a few millimeters to a complete absence. The remainder of the femoral head consisted of spongy bone. Because of problems with the separation of individual layers, in further analysis we combined samples of articular cartilage and compact bone.

Preparation of bone tissue material for analysis

Femoral heads were removed with a glass tool. Chemical analysis was performed on two materials: cartilage with directly adjacent compact bone, and spongy bone.

Bone material was dried to a constant weight at 105 °C. This procedure was used to determine water content (gravimetric method). Dried samples were ground in an agate mortar.

Determination of Mn, Fe and Sr

The crushed material was divided into samples of 0.5–1.0 g. The weighed samples were mineralized by wet digestion using a Velp Scientifica mineralizer (Italy) [25,26]. Mineralization was carried out in four step heating regime, with a maximum temperature of 200 °C. The concentrations of Mn, Fe and Sr were determined by ICP-AES (atomic absorption spectrophotometry) in inductively coupled argon plasma using a Perkin-Elmer Optima 2000 DV.

The limits of detection of the apparatus were 0.1 mg/l (Mn and Fe) and 0.05 mg/l (Sr). The results were converted into dry weight (dw).

Validation of analytical proceedings

The accuracy of the analytical procedure was monitored by determination of the studied elements in two types of reference

Table 1
Concentration of selected elements in certified reference materials in mg/kg dry wet (RW, reference value; OD, own determination).

| Metal | Bone Meal SRM NIST 1486 | | OD/RV (%) | Fish Tissue IAEA-407 | | OD/RV (%) |
|-------|-------------------------|----------------|-----------|----------------------|----------------|-----------|
| | RV | OD (n = 7) | | RV | OD (n = 8) | |
| Mn | 1.00 ^a | 0.90 ± 0.04 | 90.00 | 3.52 ± 0.32 | 3.33 ± 0.31 | 94.6 |
| Fe | 99.00 ± 8.00 | 106.00 ± 17.00 | 107.00 | 146.00 ± 14.00 | 141.25 ± 16.00 | 96.7 |
| Sr | 264.00 ± 7.00 | 226.00 ± 8.00 | 85.60 | 130.00 ± 11.00 | 115.76 ± 26.00 | 89.0 |

^a Estimated value.

Table 2
The concentration of manganese (Mn), iron (Fe) and strontium (Sr) (in mg/kg dry weight, dw) in bone of the hip joint from patients after hip replacement surgery (CACB, cartilage with adjacent compact bone; SB, spongy bone; Med, median; Q_L, lower quartile; Q_U, upper quartile; SD, standard deviation; KW, Kruskal–Wallis test; U, Mann–Whitney U-test; p, level of significance; NS, difference non-significant).

| Metal | Parameter | CACBn = 37 | SBn = 37 | KW | U |
|-------|--------------------------------|-------------|-------------|----|-------------------------|
| Mn | Med | 0.15 | 0.17 | NS | U = 526.0 (p < 0.08) |
| | Q _L –Q _U | 0.13–0.21 | 0.12–0.27 | | |
| | Mean ± SD | 0.18 ± 0.07 | 0.21 ± 0.15 | | |
| | Min–max | 0.09–0.41 | 0.02–0.8 | | |
| Fe | Med | 40.0 | 43.9 | NS | U = 518.0 (p < 0.07) |
| | Q _L –Q _U | 25.5–52.1 | 25.3–60.0 | | |
| | Mean ± SD | 43.2 ± 21.2 | 49.8 ± 33.6 | | |
| | Min–max | 13.2–107.6 | 0.64–133.8 | | |
| Sr | Med | 37.7 | 42.7 | NS | NS |
| | Q _L –Q _U | 28.1–49.7 | 28.8–58.6 | | |
| | Mean ± SD | 39.1 ± 16.0 | 42.2 ± 23.0 | | |
| | Min–max | 7.4–76.7 | 0.26–102.0 | | |

materials with known concentrations of Mn, Fe and Sr: NIST SRM 1486 Bone Meal (National Institute of Standards and Technology, USA) and IAEA-407 Trace Elements and Methylmercury in Fish Tissue (International Atomic Energy Agency, Austria). The concentration values of the reference materials given by the manufacturers and our determinations are shown in Table 1. In order to determine the possible loss of analyte during the chemical process or the impact of other factors on the results of research, we conducted recovery testing (Table 1).

Statistical analysis

Statistical studies were performed using Stat Soft Statistica 9.0 and Microsoft Excel 2007.

We calculated the arithmetic means (AM), standard deviations of the AM (SD), median (Med), lower (Q_L) and upper quartile (Q_U). In order to determine the conformity of the distribution of result with the expected normal distribution, we used a Kolmogorov–Smirnov test with Lillefors correction (p < 0.05). When the distribution of empirical data conformed with the expected normal distribution, an ANOVA test was used, and in the case of statistically significant differences, a Student's *t*-test was used. In the absence of conformity between the expected normal distribution and the empirical data, we used the nonparametric Kruskal–Wallis test, and the significance of differences was confirmed by the Mann–Whitney test. Finally a Spearman rank correlation coefficient was calculated between trace elements occurring in the material, as well as between the different metals studied in different parts of the hip joint.

Results

In the examined patient bone samples Fe concentration was the highest while Mn concentration was the lowest (Table 2). In both the spongy bone and the cartilage with adjacent compact bone, concentrations of the elements could be arranged in the following descending order: Fe > Sr > Mn.

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