AGE-RELATED DECREASES IN BONE MORPHOGENETIC PROTEIN-4 IN HUMAN DEMINERALIZED BONE

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Introduction

Demineralized bone matrix (DBM) can induce ectopic endochondral formation that can be utilized to enhance bone healing in a number of clinical applications. DBM can be prepared by acid extraction of allograft bone, leading to loss of some mineralized component but retention of collagen and noncollagenous proteins, including cytokines and growth factors. DBM possesses substantial abilities for regeneration, remodeling, and repair. It has been demonstrated that bone protein extracts implanted into small animals at heterotopic sites, induce the formation of new bone tissues. The sequential bone development cascade is reminiscent of cartilage and bone morphogenesis, and it is regulated by osteoinductive factors including bone morphogenetic proteins (BMPs) and transforming growth factor-β (TGF-β). Among proteins in the BMP family, BMP-4 is able to induce de novo chondrogenic and osteogenic formation and appears to be influential for skeletal development during mammalian embryogenesis. BMP-4 also plays a crucial role in bone remodeling and fracture repair, as demonstrated by the increase of the BMP-4 expression during fracture healing. The objective of the present study was to determine whether the extractable BMP-4 content depends upon the age and gender of the DBM donor. We postulate that variability in BMP-4 content extractable from DBM could be attributed to the age and gender of the DBM donor.

Materials and Methods

Bone samples of 63 donors (36 men between the ages of 15-65 years and 27 women between the ages of 17-65 years) were ground and demineralized by exposure to 0.5 N HCl, after which the ground demineralized bone matrices were freeze dried and stored at ~80 °C. DMB samples were extracted by collagenase digestion. Briefly, demineralized bone matrices with particle size ranging from 250-850 micron were digested with type I collagenase in 200 mM Tris-HCl buffer, pH 7.2 with 3 mM CaCl₂, 3 mM MgSO₄, 20 mM NaCl, 3 mM N-ethylmaleimide (NEM), 0.1 mM phenylmethanesulfonyl fluoride (PMSF), and 0.1 mM benzamidine-HCl at 37 °C for 24 hours with continuous shaking. The mixture was then centrifuged and the supernatant dialyzed against distilled water at 4 °C overnight. The dialysate was recovered and stored at ~20 °C until assayed for BMP quantity.

BMP-4 was measured by sandwich enzyme-linked immunosorbert assay (ELISA) using a commercially available kit (R&D Systems). Briefly, 200 µl of sample or appropriate standard were added to each well of a 96 well-microtiter plate precoated with anti-BMP-4 monoclonal antibody and incubated for 2 hours at room temperature. Following a wash to remove any unbound antibody-enzyme reagent, 200 µl of a substrate solution was added to the well and incubated for 30 minutes at room temperature. The color development was stopped with 50 µl of 2 N sulfuric acid and the intensity of the color was measured using a microplate reader at 450 nm. The concentration of BMP-4 in sample extracts was determined by comparing the optical density of the sample to a standard curve.

The data are represented as means with error bars representing standard error of the mean (SEM). Analysis of variance (ANOVA) was used to determine the significant differences among treatment groups. Tukey-type multiple comparison tests were used for comparing means of more than two groups in one way ANOVA analyses. A P value of less than 0.05 was considered to be statistically significant.

Results

It is hypothesized that donor age and gender may affect the amounts of BMP-4 extractable from DBM of similar particle size ranges and degree of demineralization. Accordingly, demineralization bone samples derived from different age groups of both male and female donors were extracted and analyzed using BMP-4 ELISA. All DBM utilized in this donor age and gender study comprised approximately 2% residual calcium and contained bone particles in the size range from 250 to 850 microns. DBM from variable donors were categorized into five age groups: 0-19, 20-29, 30-39, 40-49, and greater than 50 years. The level of BMP-4 in DBM declined by 41% in male donors (Figure 1) and by 43% in female donor (Figure 2) between the age group 0-19 years of age and 50 years of age and older. Analysis of the BMP-4 levels of the DBM samples demonstrated a significant reduction of the BMP-4 level in male donors between the 30-39 year-old age group and the 40-49 year-old age group from 3998.2 to 2746.0 pg/g of DBM (P<0.04). A similar decrease was observed in female donors between the 0-19 year-old age group and the 30-39 year-old age group, from 4712.6 to 2894.8 pg/g of DBM (P<0.05). When DBM were analyzed within these specific age ranges, the results showed an age-related decline in the bone matrix level of BMP-4 (Figure 3). The average content of extractable BMP-4 from DBM was 3248.5 pg/g of DBM. Interestingly, the BMP-4 contents in these bone samples from donors in the age group 0-19 years of age were almost two fold higher compared with the BMP-4 contents of 50 years of age and older, indicating that BMP-4 contents markedly decline during the forth and fifth decade of life (Figure 3).

Discussion

Based on the quantitative determination of BMP-4 in the DBM, this study reveals that there is an inverse relationship between the BMP-4 extractable from DBM and donor age. Three explanations could account for age-associated bone loss in terms of growth factor activities. First, the bone growth factor content could decrease as a function of aging, since the rate of bone growth factor synthesis by bone cells decreases with age. This interpretation would be consistent with our results. Secondly, the rate of production of essential binding proteins that may be responsible for the deposition of bone growth factor could decrease as a function of aging. Finally, the responses of bone cells to bone-derived growth factors decrease as a function of aging.

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