BMP-4 CONTENT IN HUMAN DEMINERALIZED BONE MATRIX CORRELATES WITH OSTEOINDUCTIVITY

*Honsawek, S; *Crouch, K; *Wilson, A; *Qin, XF; +*Wolfinbarger L
+*LifeNet, Virginia Beach, VA 23455, 757-464-4761, Fax: 757-363-2713, lloyd_wolfinbarger@lifenet.org

Introduction

Many studies have shown that Demineralized Bone Matrix (DBM) contains Bone Morphogenic Proteins (BMPs) that are essential regulators for endochondral bone formation. It is postulated that the osteoinductive potential of DBM is predominantly due to the availability of BMPs present in DBM. Among the BMP family, BMP-4 is one of the most osteoinductive factors and has been investigated for clinical application. Several in vitro bioassays have been developed and utilized for evaluation of osteoinductivity of DBM, however the results generally require at least 7 days. The in vivo athymic mouse bioassay takes even longer, 2-4 weeks. The objectives of the current study were to quantitate BMP-4 in protein extracts of DBM and investigate the relationship between the levels of extractable BMP-4 from DBM and the osteoinductivity of that DBM in the in vivo athymic mouse assay. We also examined the effect of donor age and gender on the extractability of BMP-4 from DBM.

Materials and Methods

Bone samples of 40 donors provided by LifeNet 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 ºC. DMB samples were extracted by collagenase digestion as described previously [1]. BMP-4 was measured by sandwich enzyme-linked immunosorbent assay (ELISA) using a commercially available kit (R&D Systems).

The osteoinductivities of DMB from the same donors quantitated by ELISA were assessed using the in vivo athymic mouse assay as described by Zhang [2]. DMB samples from each donor were implanted intramuscularly. After 4 weeks of implantation, the explants were isolated from the muscle pouches, fixed in 10% neutral phosphate buffered formalin, decalcified in 10% formic acid solution, embedded in paraffin, sectioned, and subsequently stained with hematoxylin and cosin. The areas of new bone and total bone (new bone and implant bone) were measured using histomorphometric analysis. The percentage of new bone formation was expressed relative to the total cross-sectional area measured.

Analysis of variance (ANOVA) was used to determine the significant differences among age groups. A Student’s t test was performed to determine the significant difference between gender groups. Correlation between BMP-4 level and osteoinductivity of DMB was analyzed by linear regression analysis (SPSS 10.00); P < 0.05 was considered to be statistically significant.

Results

An analysis of DMB from 40 donors demineralized under production conditions at LifeNet showed that the amounts of the extractable BMP-4 in the protein extracts of DMB were variable among DMB samples (Figure 1). The extractable BMP-4 levels ranged between 1.82 and 7.94 ng/g of DMB (mean = 3.70 ± 0.21 ng BMP-4/g of DMB). Figure 2 illustrates that the extractable BMP-4 levels in the protein extracts of DMB were closely correlated with the ability of human demineralized bone matrix to induce new bone formation in the nude mouse assay.

In the donor age and gender study, DMB from different donors were divided into 3 age groups for both female and male donor derived bone: 0 to 25, 26 to 44, and more than 45 year old age groups. The results of this study revealed that DMB derived from female donors contained the highest extractable BMP-4 when derived from donors in the age group 0 to 25 years of age than from donors in all other age groups tested, whereas there was no statistically significant difference in the extractable BMP-4 among DMB samples derived from male donors (Figure 3). When compared within each of age group, DMB from male donors in the age group 26 to 44 years of age and 45 years of age and older possessed more extractable BMP-4 than those from female donors in the same age group (Figure 3). However, in general, there was no statistically significant difference in the extractable BMP-4 levels of DMB between male and female donors.

Discussion

The present study demonstrated that DMB possessing high osteoinductivity in the nude mouse bioassay also contained greater BMP-4 levels in protein extracts from this DMB than those DMB samples exhibiting low levels of osteoinductivity. There was a good positive correlation between BMP-4 quantitation assay and osteoinduction (percentage of new bone formation) determined by the implantation of DMB in the athymic mouse assay with a correlation coefficient of 0.74 (p < 0.001), providing that this BMP quantitation assay could be an alternative means of assessing the capability of DMB to induce new bone formation.

References