INTRODUCTION

The periosteum is a thin layer of connective tissue that covers the surface of bones and consists of an outer "fibrous layer" and inner "cambium layer". The fibrous layer contains fibroblasts while the cambium layer contains progenitor cells which develop into osteoblasts that are responsible for increasing bone width. The presence of pluripotent mesenchymal cells in the under surface of the periosteum in combination with growth factors regularly produced or released from the surrounding tissues, provide this unique tissue with an important role in bone and cartilage healing.[1,2] Previous studies reported that during embryonic and neonatal bone growth, periosteal cells that have started to differentiate to osteoblasts show molecules characteristic of osteoblastic lineage, e.g. bone morphogenetic proteins (BMPs) and BMP receptors.[3] Although BMPs share action with a number of other molecules, all members of the TGF-β superfamily, their effects seem to be superior and more specific.[4] Despite the importance of knowledge of the properties and distribution of periosteal progenitor cells, little has been published on the characterization of human periosteal cells and how they induce or express several growth factors contributing in bone homeostasis.

The purpose of our study was to evaluate the expression of BMPs (BMP2, BMP4, BMP6) genes in human periosteum and in different subgroups, including different donor sites, smoking habits and gender.

MATERIALS AND METHODS

MATERIALS: After approval from the institutional review board, periosteal samples were obtained during reconstructive orthopaedic procedures. Periosteum samples were collected from 22 adult individuals, 12 men and 10 women (age: 18-74), 11 smokers and 11 non-smokers and from small (metatarsal) and large (humerus, radius, ulna, femur, tibia, and fibula) tubular bones.

METHODS: The main steps of the procedure were the following: Periosteum collection: Periosteum extracts were precipitated in sodium-chloride and then were processed immediately for homogenization in the presence of TRIZol Reagent (Invitrogen). RNA extraction: Was performed using the RNeasy Mini Kit (Qiagen). RNA quality was determined by electrophoresis and absorbance spectrophotometry. RT-PCR: cDNA synthesis was performed using the First Strand cDNA Synthesis kit for RT-PCR (AMV kit, Roche). Quantitative RT-PCR: BMPs (BMP2, BMP4, BMP6) mRNA levels were analysed by Quantitative Real Time-PCR. Q-RT-PCR was performed using the Light Cycler machine and the Light Cycler FastStart DNA Master Hybridization Probes (Roche). h-PBGD (porphobilinogen deaminase) was used as a control gene. Statistical analysis: the t-test for independent values was used to determine the p-value. P value < 0.05 was considered statistically significant.

RESULTS

The presence of the three BMPs (BMP2, BMP4 and BMP6) was determined in all individuals by Q-RT-PCR where BMP2 gene was determined in all individuals by Q-RT-PCR where BMP2 gene was predominately expressed. The expression of BMP2 gene was significantly higher (median: 19.77, range: 1.95) than the mRNA levels of BMP4 and BMP6 genes (median: 1.91, range: 0.2-10.2 and median: 2.36, range: 0.5-8.7, respectively) (p<0.05). Comparison of BMPs mRNA levels between small and large tubular bones has shown large variation in BMP2: the median mRNA value in large bones was 21.34 while in small ones was 14.43 (p<0.05). The median values of BMP4 and BMP6 were 1.83/1.93 and 3.07/2.19 in small and large bones, respectively. Furthermore, confrontation in a subset of 5 small tubular (metatarsals) and 7 long bones (femurs) of the lower extremity, BMP2 and BMP4 were higher in the long bones (p<0.05) whereas BMP6 levels were similar. Likewise, when periosteal samples (n=7) from the non weight-bearing bones of the upper extremity (humerus-radius-ulna) were compared to samples from the lower extremity (n=11) (femur, tibia, fibula), all BMPs mRNA expression were higher in the lower extremity samples. In particular, median value of BMP2, BMP4 and BMP6 were 11.35, 0.60, 1.46 / 27.49, 2.56, 2.62 (p<0.05) in upper and lower extremity, respectively. A gender variation was noticed in BMP2 where the median mRNA value in men was 23.36 while in women was 15.45 (p<0.05). The levels of BMP4 and BMP6 were similar between men and women (men: 1.90/women: 1.93, men: 2.54/women: 2.21, respectively) (p<0.05). In the non-smokers group all evaluated BMPs were higher compared to the smokers group, and the median mRNA levels of BMP2, BMP4 and BMP6 were 25.55, 3.05, 2.36 / 13.99, 1.72, 1.29 (p<0.05) in the non-smokers and smokers groups, respectively. Although it was not statistical significant, the values of all BMPs were almost reduced to half in smokers.

DISCUSSION

In the present study, an analysis of mRNA levels of BMP2, BMP6 and BMP4 genes in normal periosteal cells was carried out and BMP levels were compared in periosteal samples obtained from large and small tubular bones, from the upper and lower extremity, and from different gender and smoking-habits groups.

Expression of BMP2 is characteristically higher than that of BMP4 and BMP6 in our study, highlighting the role that BMP2 plays in bone homeostasis. Furthermore, the almost 2-fold reduction of all BMP-4 expression in smokers when compared to non-smokers is remarkable although not statistically significant. It is well known that smoking delays fracture union and probably the latter is correlated to the reduction of BMP expression.

Expression of all BMPs is higher in periosteal samples from weight-bearing bones of lower extremity compared to upper extremity samples. Although not statistically significant, the variation is 2- to 4-fold higher in bones of lower extremity, indicating a potential role of mechanical forces in the expression of growth factors and BMPs. Likewise, higher expression of BMP2 and BMP4 has been observed in large bones compared to small ones.

Regarding the gender, a higher expression of BMP2 was noted in males. Factors affecting osteogenic activity, such as hormones, may play a role in this discrepancy. BMPs play a highly significant role in the control of bone growth, remodelling and healing. Considering the results of our investigation it can be assumed that several parameters such as smoking habits, hormones are mechanical loads affect the expression of periosteal BMPs in each individual and specific bone.

REFERENCES

4. Shimizu T., Sasano Y. Osteoblastic differentiation of periosteum derived-cells is promoted by the physical contact with the bone matrix in vivo, The Anatomical Record (2001) 264:72-81.