A-3. The Effect of Recombinant Human Bone Morphogenetic Protei n-4 on the Osteoblastic Differentiation of Mouse Calvarial Cell s Affected by *Porphyromonas gingivalis*

Kim Chang-Sung*, Choi Seong-Ho, Choi Bong-Kyu, Chai Jung-Kiu,
Park Joon-Bong, Kim Chong-Kwan, Cho Kyoo-Sung
Department of Periodontology, Research Institute for Periodontal Regeneration, Brain Korea 21 Project for Medical Science, College of Dentistry, Yonsei University

Background: Anumber of studies have shown effective bone regeneration induced by bone morphogenetic proteins(BMPs), but it is not clear whether the presence of periodontopathic bacteria has any significant modulation effect on the bone regeneration ability of BMPs. The present study examined whether pretreatment of mouse calvarial cells with porphyromonas gingivalis extracts can make a difference in their osteoblastic differentiation exerted by recombinant human bone morphogenetic protein-4 (rhBMP-4).

Methods: Primary mouse calvarial osteoblastic(MCO) cells were cultured until they reached confluence. At confluence, cells were untreated or pretreated with 1 g/ml of sonicated P.gingivalis extracts(SPEs) for 2 days. After washing, the cells were further incubated in the presence of rhBMP-4(0-100 ng/ml) for 3 days. At the end of the treatment, the cells were harvested and lysed for measurement of the alkaline phosphatase(ALP) activity. Total RNA was extracted and reverse transcription-polymerase chain reaction(RT-PCR) analysis for expression of ALP mRNA was conducted. The amount of prostaglandin E2(PGE2) secreted into the culture supernatant wasdetermined using enzyme immuno assay.

Results: The stimulatory effect of rhBMP-4 on ALP activity was observed in both untreated MCO cells and in cells pretreated with 1 g/ml of SPEs in a dose-dependent manner. The ALP activities were significantly reduced in the cells pretreated with SPEs at all concentrations of rhBMP-4 used in this study when compared to cells untreated with SPEs. Similar results were obtained in the RT-PCR analysis for ALP mRNA. Cells pretreated with SPEs released significantly larger amount of PGE2 than untreated cells, but the treatment with 100 ng/ml of rhBMP-4 had no significant effect on the amount of PGE2 released.These results suggest that stimulatory effect of rhBMP-4 on the osteoblastic differentiation might be significantly reduced by P. gingivalis, possibly through the endogenous PGE2pathway, but rhBMP-4 still has a stimulatory effect on osteoblastic differentiation of mouse calvarial cells affected by P. gingivalis.

Conclusion: Our results suggest that supplemental BMPs would be beneficial for improved treatment of osseous defects, although their biologic effect might be significantly reduced by periodontopathic bacteria.