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RANKL-ANTAGONIST-PEPTIDE INCREASES BONE FORMATION *IN VITRO* AND *IN VIVO*

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WP9QY (W9) which was originally designed as a TNF-α-antagonist-peptide shows inhibitory effects on both TNF-α and RANKL. Our previous experiments have shown that TNF-α inhibits bone formation. But the role of RANKL in bone formation is not well clarified yet. Therefore, in this study we used OP3-4 which is a sole RANKL-antagonist-peptide and W9 peptide to elucidate the effect of RANKL on bone formation *in-vitro* and *in-vivo*. In the *in-vitro* study primary osteoblasts cells showed an increase in number of osteoblasts (osteoblastogenesis) in both OP3-4- and W9-treated cultures. Osteoblast differentiation was significantly enhanced in OP3-4 (140 μM) treated cells compared to W9 (140 μM) treated cells, which was confirmed by increased ALP staining on day 7 and bone nodule formation on day 21. For *in-vivo* bone formation, 5-week-old male C57BL/6J mice were implanted with type1 bovine-collagen containing BMP-2 (1 μg), BMP-2+W9 (0.56 mg), and BMP-2+ OP3-4 (0.28mg/0.56mg) into the back muscle of the mice. Mice were sacrificed on day 12 after implantation. Micro-CT analysis revealed that the largest size of ectopic bone was observed in BMP-2 with OP3-4 (0.56 mg) group among the experimental groups. Quantitative analysis by dual-energy X-ray absorptiometry (DXA) confirmed above observations. OP3-4 (0.56 mg) and W9 (0.56 mg) increased the bone mineral content of BMP-2-induced ectopic bone up to 4.1 ($p<0.005$) and 2.2 ($p<0.005$) times compared to BMP-2 alone, respectively. To further clarify the bone formation effect of these peptides, gelatin hydrogels containing BMP-2, W9 only (0.56 mg), and OP3-4 only (0.28mg/0.56mg) were placed on calvarial defects of mice and allowed to heal for 4 weeks. Micro-CT reconstruction images revealed no healing in control groups with or without gelatin hydrogels. Defects of OP3-4 (0.56 mg)-treated mice were fully repaired whereas OP3-4 (0.28 mg) and W9 (0.56 mg)-treated groups showed a partial repair of the defects. Confirming the Micro-CT data, DXA showed increased bone mineral density in OP3-4 and W9-treated animals. Taken together the present study shows that OP3-4 could increase bone formation more than W9. Since the affinity of OP3-4 to RANKL is more than that of W9, the acceleration of bone formation by OP3-4 might be mainly due to the inhibition of RANKL suggesting that RANKL has an anti-anabolic effect in bone.