

5kDa Component of Enamel Matrix Derivative Possesses Osteogenic Properties

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Statement of Purpose: Amelogenin is the principal constituent of enamel matrix derivative (EMD), an extracellular matrix protein complex derived from fetal porcine tooth germs, commercially known as Emdogain® (Institut Straumann AG). Diverse studies attribute the bioactive component of EMD to the complete amelogenin protein, whereas other studies indicate that the bioactive effect is due to amelogenin fragments present in the EMD (1,2). Analysis of EMD by high performance liquid chromatography revealed the presence of three main components: (1) a 20 kDa protein [Fraction A], (2) two proteins of 12 and 9 kDa, and (3) a 5 kDa peptide [Fraction C]. Two of these components (Fraction A and C) have been purified and characterized. The Fraction A protein corresponds to the full length amelogenin protein and the Fraction C is the N-terminal part of this protein (3). The aim of the present study was to examine the effect of these two EMD components on osteoblasts.

Methods: Confluent cultures of MG63 human osteoblast-like cells and normal human osteoblasts were treated with or without EMD, recombinant human amelogenin (rhAmel), Fraction A (0.01-100µg/ml) or Fraction C (0.1-250µg/ml) for 24 hours. Effects on DNA content and alkaline phosphatase specific activity (ALP), and osteocalcin (OCN), osteoprotegerin (OPG), vascular endothelial growth factor A (VEGF-A) and fibroblast growth factor-2 (FGF-2) levels in the conditioned media were determined.

Results: Fraction C reduced DNA content of MG63 cells in a dose-dependent manner and increased osteoblast differentiation markers like alkaline phosphatase and osteocalcin with peak increases at 10 mg/ml. The peptide also increased local factors like OPG, VEGF and FGF in a dose-dependent manner. The effects of the Fraction C were similar to those of the Fraction A, EMD, and rhAmel. Moreover, normal human osteoblasts responded in a similar manner to MG63 cells.

Conclusions: These results indicate that the Fraction C component of Emdogain possesses osteogenic activities and that the osteogenic effects of amelogenin may be due the N-terminal region of the protein.

References:

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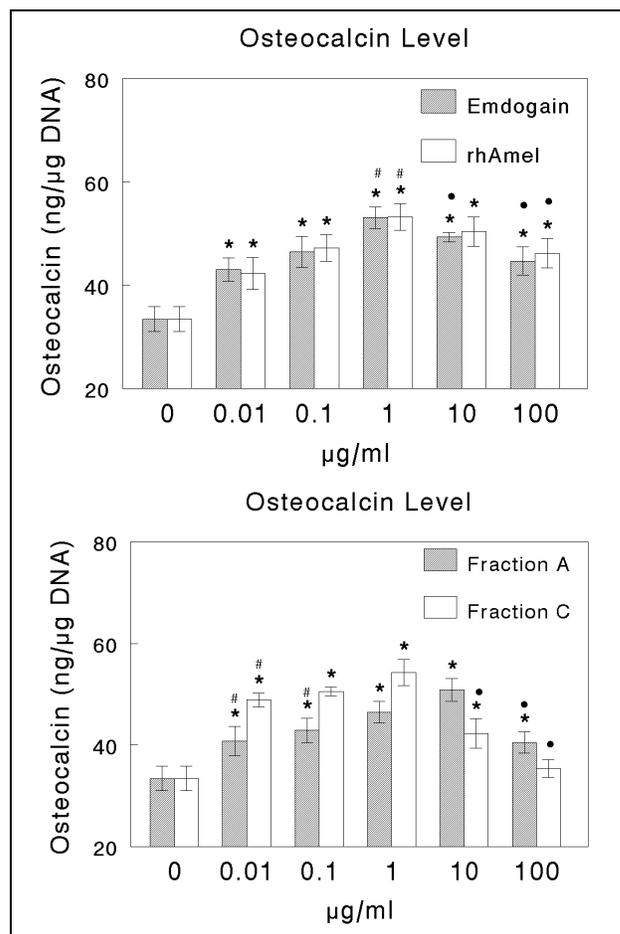


Figure 1. Top Panel: EMD and rhAmel caused a biphasic increase in osteocalcin production. Bottom Panel: Similarly Fraction A and Fraction C had a biphasic effect. For EMD, rhAmel and Fraction A, maximal increases were at 1 µg/ml and for Fraction C, maximal increases were at 10 µg/ml. Data are from one of two experiments and are means \pm SEM for N=6 independent cultures per variable. Both experiments showed similar results. * $p < 0.05$. treatment v. control.