Background: The repair / regeneration of non-union bone fractures and regeneration of mineralized mesenchymal tissues affected by chronic diseases are a major scientific and clinical challenge. One such prevalent chronic disease is characterized by the destruction of the tooth supporting tissues: alveolar bone, cementum and periodontal ligament (PDL), which sometimes leads to tooth loss. We have shown in the past that the recombinant human amelogenin protein (rHAM+) (Taylor et al. 2006) alone, produced in the eukaryotic system, brought about in vivo regeneration of the periodontal tissues, after induction experimental of periodontitis, in the dog model, through recruitment of mesenchymal stem cells (Haze et al. 2009). Amelogenin is expressed in bone cells, bone marrow cells and cartilage.

Research Hypothesis: rHAM+ can repair in vivo non-union defects in the calvarial (membranous) bone and long (endochondral) bone.

Aims: Regeneration of non-union defects of calvarial bone and long bone, in vivo, in the mouse model using rHAM+

Methods: (A) Creation of critical size defect in parietal (calvarial) bone. (B) Application of rHAM+ to the calvarial bone defect. (C) Characterization of the regenerated bone tissue by micro-CT analysis, histological, immunological and molecular biology technologies. (D) Creation of non-union segmental fracture in mouse radial (long) bone, and analyses of long bone regeneration. (E) Studying whether the regeneration involves recruitment of mesenchymal stem cells

Results: The extent of in vivo regeneration of non-union calvarial defect (membranous bone), depends on amelogenin concentration and time of regeneration. The newly formed bone, which bridges the edges of the defect, is of the same thickness and has the same histological characteristics as normal (un-operated) bone, while at times sporadic tissue, not bridging the edges of the defect was produced without application of amelogenin. Amelogenin expression, as well as the expression of CD105 (marker of mesenchymal stem cells), BSP, osteocalcin and TGFβ (markers of bone), are elevated in the regenerated bone, as compared to normal (un-operated) bone, and control (operated bone with no application of amelogenin). Statistical analysis of the regenerated bone using rHAM+ as compared to control was close to statistical significance (P=0.067). Preliminary studies using micro-CT analysis of the regeneration of non-union long bone fractures (endochondral) revealed that rHAM+ can also bring about regeneration of long bone.

Discussion: Amelogenin induces regeneration according to the specific niche; when added to periodontal defect amelogenin caused the regeneration of all three periodontal tissues, in the correct order – first cementum and periodontal ligament and only later –bone, while only bone was formed in the calvarial and long bone defect. The regenerated calvarial bone resembles normal bone; it has normal thickness, structure and bone markers. Increasing sample size is required to achieve statistical significance.

Conclusions: The recombinant human amelogenin rHAM+ can regenerate non-union defects in the calvarial (membranous) bone and preliminary results also indicate its ability to regenerate long (endochondral) bone.

Key words: Repair, Calvariae, Long bone, Non-union fracture, Recombinant amelogenin.
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