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ScienceDirect

Journal of the Chinese Medical Association xx (2018) 1−9

www.jcma-online.com

Original Article

Parathyroid hormone gene-activated matrix with DFDBA/collagen composite matrix enhances bone regeneration in rat calvarial bone defects

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Received October 30, 2017; accepted November 10, 2017

Abstract

Background: Gene-activated matrix (GAM) induces sustained local production of growth factors to promote tissue regeneration. GAM contains a plasmid DNA (pDNA) encoding target proteins that is physically entrapped within a biodegradable matrix carrier. GAM with a pDNA encoding the first 34 amino acids of parathyroid hormone (PTH 1−34) and a collagen matrix enhances bone regeneration in long bone defects. Demineralized freeze-dried bone allograft (DFDBA) is a widely used osteoinductive bone graft. The present study determined the osteogenic effects of PTH-GAM with a collagen or DFDBA/collagen composite (D/C) matrix for treating craniofacial bone defects.

Methods: We constructed a pDNA encoding human PTH 1−34 and performed cyclic AMP ELISA to verify the bioactivity of PTH 1−34. Next, we generated a D/C matrix and PTH-GAMs containing a collagen matrix (PTH-C-GAM) or D/C matrix (PTH-D/C-GAM). Rats with critical-sized calvarial bone defects were divided into four groups, namely, untreated rats (sham group) and rats grafted with D/C matrix, PTH-C-GAM, or PTH-D/C-GAM (D/C, PTH-C-GAM, or PTH-D/C-GAM groups, respectively). PTH expression was determined by performing immunohistochemical staining after 4 and 8 weeks. New bone formation was evaluated by performing radiography, dual-energy X-ray absorptiometry, microcomputed tomography, and histological examination.

Results: PTH pDNA-transfected cells secreted bioactive PTH 1−34. Moreover, PTH was expressed at 4 and 8 weeks after the surgery in rats in the PTH-C-GAM group but not in rats in the D/C group. New bone formation in the calvarial bone defects, from more to less, was in the order of PTH-D/C-GAM, PTH-C-GAM, D/C, and sham groups.

Conclusion: Our results indicate that PTH-GAM with a collagen matrix promotes local PTH production for at least 8 weeks and bone regeneration in craniofacial bone defect. Moreover, our results indicate that replacement of the collagen matrix with the D/C matrix improves the osteogenic effects of PTH-GAM.

Keywords: Allografts; Bone morphogenetic proteins; Bone regeneration; Gene therapy; Parathyroid hormone

1. Introduction

Gene-activated matrix (GAM) technology, a non-viral gene therapy strategy, promotes sustained local production of growth factors to enhance tissue regeneration.[1] GAM contains a plasmid DNA (pDNA) encoding target proteins that is physically entrapped within a biodegradable matrix carrier. During the healing process, cells migrate into the matrix, take up the pDNA, and express the encoded proteins. Although the

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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https://doi.org/10.1016/j.jcma.2017.12.004

GAM technology is an economic, safe, and simple strategy, the transfection efficiency of GAM is low. Polycations such as polyethylenimine (PEI) have been used to condense pDNA, facilitate its entry into cells, increase gene expression, and enhance the osteogenic effect of GAMs.[2,3] GAMs with pDNA encoding various osteogenic factors and with different matrices have been studied for promoting bone regeneration.[1,2,4−8]

Parathyroid hormone (PTH), a polypeptide containing 84 amino acids (PTH 1−84), regulates blood calcium levels by exerting catabolic effect on bones. Intriguingly, PTH and some PTH fragments exert anabolic effects on bones under certain conditions.[9] PTH 1−34, which contains 34 amino acids of the N-terminus of PTH, exerts anabolic effects on bones.[10,11] PTH 1−34 binds to PTH receptors on osteoblasts and activates several signaling pathways. The anabolic effects of PTH on osteogenic cells are mainly mediated by the activation of cyclic AMP/protein kinase A (cAMP/PKA) signaling pathway.[12]

PTH 1−34 greatly enhances bone formation. Systemic PTH 1−34 administration through a low-dose intermittent subcutaneous injection increases bone density in osteoporosis,[13,14] enhances bone healing,[15] and promotes allograft integration[16] in rats with calvarial bone defects. Local delivery of PTH 1−34 along with an RGD peptide by using a hydrogel matrix exerts beneficial effects on bone defect healing around implants.[17] Local application of PTH 1−34 by using a membrane effectively enhances the healing of 5 mm calvarial bone defect in rats.[18] The GAM technology has been used to deliver PTH 1−34 for promoting bone regeneration in long bone defects.[1,19,20] However, the effect of PTH-GAM for treating craniofacial bone defects is still unclear.

Demineralized freeze-dried bone allograft (DFDBA) is one of the most widely used bone grafts in dentistry. DFDBA contains type I collagen, bone morphogenetic proteins (BMP-2, BMP-4, and BMP-7), and other growth factors (including FGF-1 (fibroblast growth factor-1), IGF-1 (insulin-like growth factor-1), TGF-β1 (transforming growth factor-beta1), VEGF (vascular endothelial growth factor) and PDGF (platelet-derived growth factor)).[21,22] The osteoinductive property of DFDBA is attributed to the exposure of its inherent BMPs through demineralization.[23] A DFDBA/collagen composite (D/C) matrix has been used for improving the handling properties of powdered DFDBA. Furthermore, this matrix is more effective than DFDBA[24] or collagen[25] for promoting bone regeneration.

Several studies indicate that PTH 1−34 and BMPs exert synergistic effects for promoting bone regeneration. Combined use of intermittent systemic PTH 1−34 and BMP-2 induces higher bone formation than the use of PTH or BMP alone in a rat model of spinal fusion.[26] Similarly, combined use of intermittent systemic PTH 1−34 and BMP-7 exerted a synergistic effect in a rabbit model of metaphyseal bone healing.[27] Furthermore, intermittent systemic PTH 1−34 treatment improves BMP-2-induced ectopic and orthotopic bone formation.[28] Dual delivery of PTH 1−34 and BMP-4 by using a two-plasmid GAM promotes better bone healing than individual delivery of PTH 1−34 or BMP-4 by using GAM.[19]

Local sustained delivery of osteogenic factors through regional gene therapy may enhance bone defect healing with reduced systemic toxicity and prevent the requirement of supraphysiological doses of therapeutic proteins.[1,29] PTH-GAM encoding PTH 1−34 and with a collagen matrix promotes the healing of long bone defects.[1,19] Long bones are formed through endochondral ossification, whereas craniofacial bones are formed through intramembranous ossification. Therefore, therapeutic effects of PTH-GAM for treating craniofacial bone defects should be elucidated. We hypothesized that PTH-GAM with a collagen matrix promoted bone regeneration in craniofacial bone defects and that the use of PTH-GAM with a D/C matrix exerted better anabolic effects than PTH-GAM with a collagen matrix. Thus, the present study determined the effects of PTH-GAM with a collagen or D/C matrix in a rat model of critical-sized calvarial bone defects.

2. Methods

2.1. Experimental design and timelines

The study design is shown in Fig. 1. A gene vector encoding human PTH 1−34 was prepared, and cAMP ELISA was performed to determine the bioactivity of PTH 1−34 secreted by cells transfected with this vector. Materials for implantation into the rat model of calvarial bone defects, including D/C matrix, PTH-GAM with a collagen matrix (PTH-C-GAM), and PTH-GAM with a D/C matrix (PTH-D/C-GAM), were prepared. The structure of PTH-C-GAM was examined by performing scanning electron microscopy (SEM). Rats with critical-sized calvarial bone defects were divided into four groups, namely, untreated rats (Sham group) and rats grafted with D/C, PTH-C-GAM, and PTH-D/C-GAM (D/C, PTH-C-GAM, and PTH-D/C-GAM groups, respectively). Endpoint analyses were performed after 4 and 8 weeks. PTH expression was determined by performing immunohistochemical staining, and bone regeneration was evaluated by performing radiography, dual-energy X-ray absorptiometry (DEXA), microcomputed tomography (μCT), and histological examination (H&E staining).

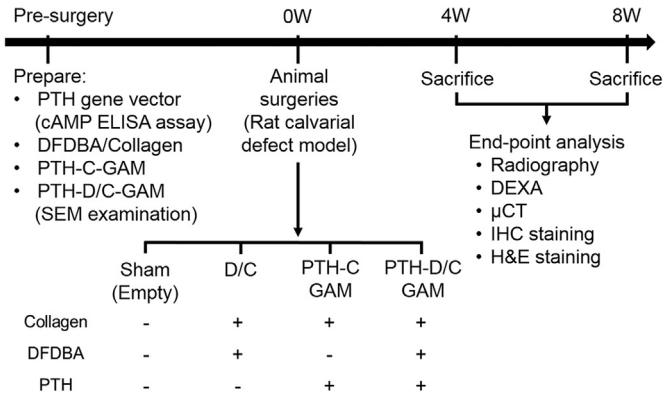

Fig. 1. **Study design and timeline**. SEM = scanning electron microscopy; DEXA = dual-energy X-ray absorptiometry; IHC staining = immunohistochemical staining.

Please cite this article in press as: Lee P-H, et al., Parathyroid hormone gene-activated matrix with DFDBA/collagen composite matrix enhances bone regeneration in rat calvarial bone defects, Journal of the Chinese Medical Association (2018), https://doi.org/10.1016/j.jcma.2017.12.004