THE OSTEOINDUCTIVITY OF ADENOVIRAL BMP2 EX VIVO GENE THERAPY IN COMBINATION WITH VARIOUS COLLAGEN CARRIERS

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INTRODUCTION: The concept of endogenous BMP2 induction by adenoviral gene therapy has emerged as a viable alternative to the delivery of exogenous rhBMP (1). The delivery of cells transduced ex vivo with adenovirus encoding BMP2 has the potential of rapidly achieving a high concentration of endogenous BMP2, and providing an efficient method of osteoinduction (2,3). However, an optimal method for endogenous adenoviral BMP2 production, which is both safe and efficacious, remains to be established. The exogenous delivery of rhBMP requires a suitable carrier to localize and control BMP releasing kinetics. The principle role of the carrier is to ensure the availability of active BMP for the target cells. Among the carrier materials employed for exogenous BMP delivery, bovine collagen has become the most prevalent. Similarly, an endogenous BMP2 delivery system consisting of adenovirally-transduced cells seeded on a suitable carrier/scaffold could potentially augment the cellular ability to express and release BMP2. Furthermore, the biological and biomechanical interactions of the transduced cells-carrier/scaffold system with the host cells post implantation could potentiate the in vivo effects of endogenous BMP, and the osteoinductivity of the system. However, the merits of using carriers/scaffolds for endogenous BMP2 delivery are unknown.

OBJECTIVES: To determine the effects of various collagen carriers/scaffolds on the ability of human mesenchymal stem cells transduced ex vivo with a novel chimeric adenovirus ad5f35-BMP2 to induce heterotopic bone. The in vitro release and bioactivity of BMP2 was also assessed and compared with that of rhBMP2. Furthermore, the nature and extent of heterotopic bone was analyzed and compared in the absence and presence of two types of collagen carrier/scaffold systems. MATERIAL & METHODS: The primary target cells for adenoviral transduction were human mesenchymal bone marrow cells (BMCs) derived from discarded bone marrow transplants donated by healthy donors following the approval of the Intuitional Review Board. The vector included a novel chimeric adenovirus type 5 carrying fiber from adenovirus 35 encoding BMP2 (ad5f35-BMP2). The construction, characteristics and transduction efficiency of Ad5f35 have been described elsewhere (4,5). The adenovirus with empty cassettes (ad5f35-HM4) was used as a control. The carriers/scaffolds were two structurally dissimilar bovine type I collagen systems of equal volumes: a viscous injectable collagen gel and a porous collagen sponge. Both carriers/scaffolds have been cleared by the FDA for clinical use, and are currently clinically available as the Zygdom II (INAMED, Santa Barbara, CA) and InFuse (Medtronic -Sofamor Danek, Memphis TN), respectively. In vitro, the ad5f35-BMP2 expression was identified, quantitated, and its activity compared with rhBMP2. In vivo, the osteoinductivity of ad5f35-BMP2 was assessed heterotopically using non-obese diabetic severe combined immunodeficiency (NOD/SCID) mice. The animals were injected intramuscularly in both hind limbs with a solution containing cells transduced ex vivo with the specific adenovirus without scaffold (Group I), seeded within the collagen gel (Group II), or seeded within the collagen sponge (Group III). Animals were euthanized 14 days post implantation. The nature and extent of heterotopic bone formation were analyzed radiographically using plain X-ray and microCT (MicroPhotonics, Inc. Allentown, PA) and histologically. The human origin of the cells in the injection site was traced using anti-human mitochondrial protein assay. Statistical analysis of the parametric data (n=6) was done with ANOVA.

RESULTS: BMCs transduced with ad5f35-BMP2 secreted considerable amount of BMP2 as determined by Western blot for the experimental groups. The biological activity of the in vitro produced ad5f35-BMP2 is depicted in Fig.1. No significant differences were observed between the Groups in the levels and/or activity of in vitro released BMP2. In vivo, heterotopic bone was consistently formed in all experimental groups at 14-days (Fig.2). No heterotopic bone was detected radiographically and histologically in all control ad5f35-HM4 groups. Quantitative microCT analysis of the heterotopic bone demonstrated a uniform distribution of trabecular thicknesses in all Groups; whereas the presence of a collagen scaffold (Group II, III) resulted in less uniform trabecular separation (bone porosity) compared to Group I. Histological evaluations corroborated the plain radiographic results. Significant amounts of new enchondral bone were consistently formed in all animals treated with ad5f35-BMP2, irrespective of the absence or presence of a collagen carrier/scaffold. In the Group I (no scaffold), the heterotopic bone permeated between the muscle fibers while radiating outward and/or splitting muscle layers. In Group II (collagen gel) and III (collagen sponge), the new bone formation did not exceeded the boundaries of the scaffold. Cross-sectional analysis of the Group III demonstrated denser bone at the periphery as compared to the core of the carrier/scaffold. The newly formed bone in Groups I and II was entirely host (murine) origin (human antigens absent); whereas new bone in Group III demonstrated the presence of human BMCs (human antigens present) within the scaffold.

DISCUSSION: Efficient osteoinduction has previously been reported using endogenous ad5f35-BMP2 adenoviral delivery systems without a carrier/scaffold. This study demonstrates that the presence of the collagen scaffold does not significantly affect the extent of ad5f35-BMP2-induced osteogenesis. The presence of a collagen carrier/scaffold for endogenous ad5f35-BMP2 production localizes osteogenesis to the region of the carrier/scaffold. The prospects of using various forms of collagen carriers/scaffold for ad5f35-BMP2 delivery could expand the indications and modes of delivery for this method.


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Fig. 1.

![Bioactivity of In Vitro Released ad5f35-BMP2 vs ad5f35-HM4](image)

Fig. 2.

![Graphs showing bioactivity](image)