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Optimal condition of heparin-conjugated fibrin with bone morphogenetic protein-2 for spinal fusion in a rabbit model

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Abstract

Background aims. Heparin-conjugated fibrin (HCF) is a carrier for long-term release of bone morphogenetic protein-2 (BMP-2) and has been shown to promote bone formation in animal models. We performed an experimental study to determine the optimal dose of BMP-2 with an HCF carrier that promotes bone formation comparable to that of autograft while minimizing complications in spinal fusion. *Methods.* Twenty-four rabbits underwent posterolateral fusion of the L5–6 spinal segments. Different concentrations of HCF BMP-2 (1/10, 1/20, 1/30 or 1/40) were implanted in the spines of experimental rabbits, and autograft or INFUSE was implanted in the spines of control animals. Eight weeks after treatment, spinal fusion efficacy was evaluated by plain radiography, micro-computed tomography (micro-CT), mechanical testing and histomorphometry. *Results.* Similar to autograft, the 1/40 HCF BMP-2 showed significant bone formation on micro-CT and histomorphometry with mechanical stability. However, the other HCF BMP-2 concentrations did not show significant bone formation compared with autograft. Although conventional BMP-2 (INFUSE) led to higher bone formation and stability, it also led to excessive ectopic bone and fibrous tissue formation. *Conclusions.* This study suggests the optimal concentration of BMP-2 using HCF for spinal fusion, which may decrease the complications of high-dose conventional BMP-2.

Key Words: *autograft, BMP-2, heparin-conjugated fibrin, spinal fusion*

Introduction

Bone morphogenetic proteins (BMPs) are osteoinductive growth factors that offer significant therapeutic promise for bone regeneration. Two BMPs (BMP-2 and BMP-7) are currently available for clinical use in lumbar fusion surgery (1,2). However, high doses of BMPs that are administered to bone defects can cause side effects, such as ectopic bone formation and various immune reactions (3). Overcoming these problems requires identifying the appropriate delivery system that minimizes the BMP dose while promoting functional improvement. Recently, heparin-conjugated fibrin (HCF) gel has been highlighted as an alternative carrier for BMP-2 delivery (4–8). Several authors have reported that HCF gel can achieve a sustained release of BMP-2 and promote the osteogenic efficacy of BMP-2 in various animal models. However, no studies have compared how different doses of BMP-2 affect the therapeutic outcomes, and questions remain regarding protein dose-response relationships. Therefore, the aim of the present study was to characterize and evaluate the dose-response of BMP-2 in an HCF delivery system on bone regeneration for lumbar spine fusion and to assess whether the results were comparable to those of auto-graft.

Methods

Subjects

Twelve-week-old New Zealand white rabbits weighing 2.5–3.0 kg were used for this study. The rabbits were divided into four groups of four rabbits each, and each group received a different dose of heparin-conjugated BMP-2. An additional eight rabbits served as controls and had either autogenous iliac chip bone grafting or conventional BMP-2 grafting (INFUSE, Medtronics, Memphis, TN, USA;

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Table I. Implant assignment of the groups in the study.

Group	Total grafts	Harvested 4 weeks	Harvested 8 weeks	BMP-2 concentration (mg/mL)	Ratio to conventional BMP-2
1	8	2	6	—	Autograft
2	8	2	6	0.15	1:10
3	8	2	6	0.075	1:20
4	8	2	6	0.05	1:30
5	8	2	6	0.037	1:40
6	8	2	6	1.5	1:1 (INFUSE)

Table I). Posterolateral lumbar spinal fusion was then performed with the graft material in all 24 rabbits. This protocol, including animal care and use, was approved by the Institutional Committee for Animal Care and Experiments.

Synthesis of heparin conjugated fibrin (HCF)

HCF was fabricated as previously described (7). In brief, heparin (molecular weight 4000–6000 Da; Sigma-Aldrich, St. Louis, MO, USA) was covalently bonded to plasminogen-free fibrinogen (Sigma-Aldrich) by using standard carbodiimide chemistry. Heparin (100 mg) was dissolved in a buffer solution (100 mL, pH 6) of 0.05 mol/L 2-morpholinoethanesulfonic acid (Sigma-Aldrich). N-hydroxysuccinimide (0.04 mmol/L; Sigma-Aldrich) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (0.08 mmol/L; Sigma-Aldrich) were added to the solution to activate the carboxylic acid groups of the heparin. After 12 h of reaction at 4°C, the solution was stirred to obtain a homogeneous solution, and the product was precipitated with excess anhydrous acetone and lyophilized. Fibrinogen (100 mg) was dissolved in phosphate-buffered saline (20 mL, pH 7.4) without bubbles at 4°C and reacted with activated carboxyl acid groups of the heparin (60 mg) under the same conditions for 3 h. The product was precipitated with a large excess of acetone and lyophilized. The resultant white powder was completely dissolved in phosphate-buffered saline and dialyzed through a porous membrane bag (12,000–14,000 Da molecular weight cutoff; Spectrum Lab, Rancho Dominguez, CA, USA) to remove residual heparin at 4°C for 24 h. Finally, HCF was lyophilized for 48 h.

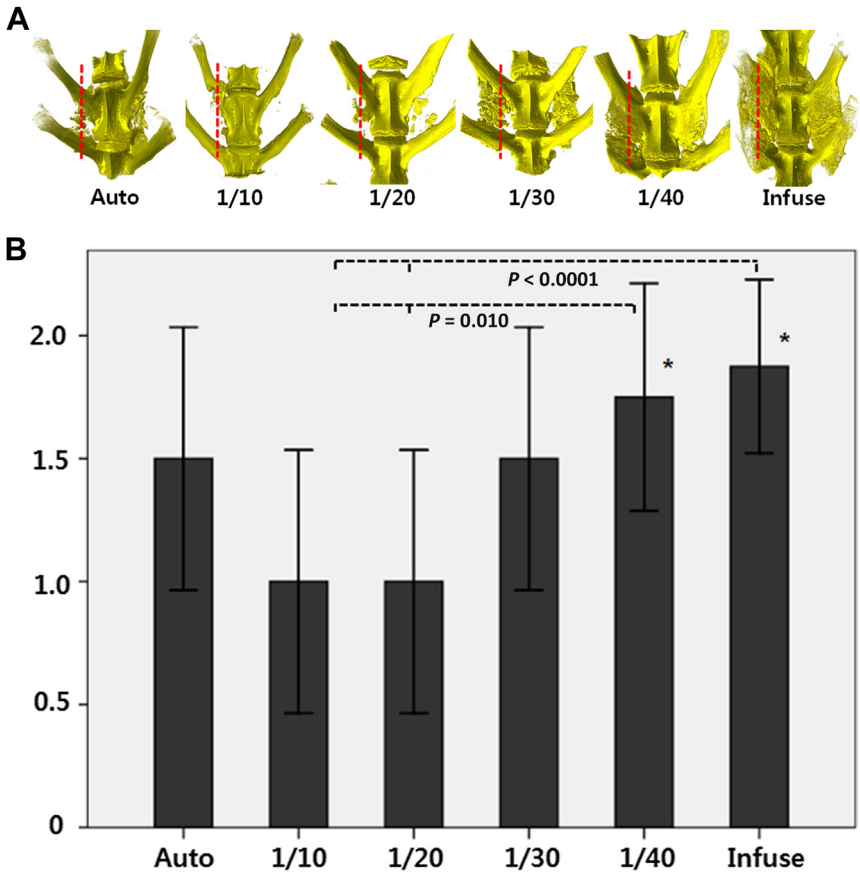

Figure 1. (A) Representative three-dimensional CT images of posterolateral fusion at 8 weeks after surgery. The fusion masses of the 1/40 BMP-2 and INFUSE groups were well remodeled. (B) Radiological fusion scores of the 1/40 BMP-2 and INFUSE groups were higher than those of the other groups, and both scores were comparable to those of the autograft group. Values are mean ± SD.