



Brief communication

Inhibition of endogenous antagonists with an engineered BMP-2 variant increases BMP-2 efficacy in rat femoral defect healing

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ABSTRACT

Bone morphogenetic proteins (BMP) have been used successfully by orthopedic clinicians to augment bone healing. However, these osteoinductive proteins must be applied at high concentrations to induce bone formation. The limited therapeutic efficacy may be due to the local expression of BMP antagonists such as Noggin that neutralize exogenous and endogenous BMPs. If so, inhibiting BMP antagonists may provide an attractive option to augment BMP induced bone formation. The engineered BMP-2 variant L51P is deficient in BMP receptor type I binding, but maintains its affinity for BMP receptor type II and BMP antagonists including Noggin, Chordin and Gremlin. This modification makes L51P a BMP receptor-inactive inhibitor of BMP antagonists. We implanted β -tricalcium phosphate ceramics loaded with BMP-2 and/or L51P into a critical size defect model in the rat femur to investigate whether the inhibition of BMP antagonist with L51P enhances the therapeutic efficacy of exogenous BMP-2. Our study reveals that L51P reduces the demand of exogenous BMP-2 to induce bone healing markedly, without promoting bone formation directly when applied alone.

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1. Introduction

Recombinant bone morphogenetic proteins such as BMP-2 and BMP-7 have been approved for clinical use to promote bone healing in tibia fractures, long bone non-unions and spinal fusions [1,2]. The growth factors, however, must be administered at concentrations that exceed those of naturally occurring BMPs present in 1000 kg of bone tissue [3]. This may be due to the *in vivo* regulation of the bioavailability of endogenous BMP by antagonistic proteins such as Noggin and members of the Dan and Chordin families [4,5]. Furthermore, BMPs themselves modulate the expression of various BMP regulatory proteins, including Noggin, Gremlin and Crossveinless-2 [6–8].

Expression of BMP antagonists increases during fracture healing and distraction osteogenesis [9–11]. Therapeutic requirements for administering high BMP concentrations to induce bone formation *in vivo* may result from significant neutralization of the growth factor by BMP inhibitory proteins. Thus, for clinical applications, the inhibition of BMP antagonists, rather than the direct application of BMPs, may present a preferred future strategy. L51P is an *in vitro* engineered BMP-2 variant with a leucine-to-proline substitution at codon 51. The modified protein is deficient in BMP type I receptor activation, but maintains its affinity for BMP type II receptors and inhibitory proteins such as Noggin, Gremlin and Chordin [12]. At equimolar concentrations, L51P blocks the inhibitory action of Noggin and restores BMP-2 dependent induction of alkaline phosphatase *in vitro* [12]. However, when added alone, L51P does not induce the differentiation of ATDC5 and C2C12-cells. Therefore, L51P represents a tool to enhance BMP-2-mediated bone formation *in vivo* through inhibition of endogenous BMP antagonists. Here, we determined whether the amount of BMP-2 required to induce bone formation could be reduced in the presence of L51P in a critical-size segmental defect in rat femur.

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2. Methods

Ceramic β -tricalcium phosphate (β -TCP) cylinders (5 mm in diameter, 6 mm in length, 75% porosity) were provided by the Robert Mathys Foundation (RMS), Bettlach, Switzerland. Wistar rats (female, retired breeders, 350–450 g), purchased from Charles River (Sulzfeld, Germany), were housed in the Central Animal Facility of the Medical Faculty, University of Bern, Switzerland, in compliance with the Swiss Federal Government guidelines for care and use of experimental animals. The Bernese State Committee for the Control of Animal Experimentation approved our study

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