

BMP-ANTAGONIST NOGGIN INHIBITS BONE REGENERATION

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Relevance to musculo-skeletal conditions: This paper establishes the role of endogenous BMPs in bone regeneration.

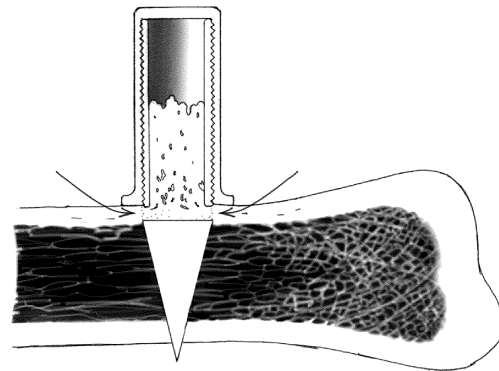
Introduction: The secreted polypeptide Noggin is a potent BMP-antagonist which binds with high affinity to BMP2 and BMP4 and blocks their association with their cognate receptors, thus prohibiting BMP/receptor interaction. Although initially characterized as a dorsalizing activity expressed in the Spemann organizer during gastrulation, mammalian Noggin has been shown to play a fundamental role in normal bone development. Noggin is highly conserved and differs by only one amino acid between human and mouse.

The physiological role of endogenous BMPs in bone regeneration is still unclear. For exogenous BMPs to stimulate bone healing, such large doses are needed that it could be speculated that endogenous BMPs are unimportant. We investigated if an exogenous Noggin could inhibit bone regeneration, because if so, this would finally establish the role of endogenous BMPs in bone regeneration.

Material and Methods: In short, Noggin was applied to collagen sponges inside titanium bone chambers, and its effect on bone ingrowth into the chamber was studied histologically (figure 1).

The bone conduction chamber (BCC) consists of a screw with a cylindrical interior space, which has holes for tissue ingrowth only at one end. Thus, the tissue ingrowth distance from the holes towards the other end of the chamber can be measured as an estimation for tissue regeneration. A genetically engineered recombinant human Noggin mutein, hNG• B2-Fc, which exhibits superior pharmacokinetic properties to that of wild-type Noggin was produced in CHO cells and purified to homogeneity (>99% purity). A volume of 16 μ l was applied to collagen sponges (HelistatTM) that would fit in the chambers, corresponding to either 1 or 10 μ g Noggin per chamber. The sponges were then lyophilized. Two groups of 10 male Sprague Dawley rats were used. Each rat received bilateral BCCs: one for Noggin and one for the sponge with correspondingly diluted buffer only. One group received 1 μ g and the other 10 μ g Noggin. The rats were sacrificed after 4 weeks. An intravenous injection of 7.3 Mbq ^{99m}TcHDP was given 3 h before sacrifice of the 10 μ g group, and the scintimetric activity of the harvested tissue was measured in a well counter. The specimens were then prepared for decalcified histology with sections parallel to the long axis of the chamber. Histological and histomorphometric assessment was performed blindly. The area of the ingrowing tissue in general and of the bone was measured by circumscribing it on a digitizing table. The ingrowth distance was calculated by dividing the area with the width of the specimen. Results were analyzed by Student's paired t-test.

Figure 1: The Bone Conduction Chamber. Implant in position at the proximal tibial metaphysis. The ingrowth holes (arrows) are situated in bone. All new ingrown tissue is shown white in the chamber, only the lower part is new bone.



Results: 1 μ g: Total tissue ingrowth was less than normal in this type of chamber in both Noggin and controls. Carrier collagen was still in place and had apparently disturbed tissue ingrowth. Upon check it was found that a collagen sponge with a slightly different production process had been delivered to us by mistake. This led to slower resorption. However, in the Noggin chambers bone formation was reduced by 17 % compared to controls ($p=0.03$). Total tissue ingrowth did not differ.

10 μ g: This group was done with the fast resorbing collagen sponge (after the mistake above had been corrected), and tissue ingrowth appeared undisturbed. The total tissue ingrowth distance was 3.1mm for Noggin and 3.0 for controls (ns). Bone ingrowth was 1.0 for Noggin and 1.5 for controls (31% reduction; $p=0.003$). The ^{99m}TcHDP uptake was reduced by 29% ($p=0.002$).

Discussion: The inhibition is most likely to be caused by Noggin blocking the endogenous BMPs in the regenerating tissue. This was a specific effect, in that bone differentiation was inhibited, but not tissue ingrowth in general. The histological picture gave no indication for infection or any immunological reaction, and the high purity of the material also makes unspecific inhibitory effects less likely. The results are in accordance with a role for Noggin in skeletal development, as has recently been shown by knocking out Noggin in mice: this led to severe skeletal malformations¹. Not only BMP expression, but also a balance between various BMPs and BMP inhibitors may account for skeletal development as well as fracture healing and implant fixation.

Reference:

1. Brunet LJ et al. Science 1998 May 29; 280(5368): 1455-1457
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** Regeneron inc. New York, NY

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