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Treatment with recombinant human bone morphogenetic protein 7 leads to a transient induction of neutralizing autoantibodies in a subset of patients

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ABSTRACT

Background: Recombinant human bone morphogenetic protein 7 (rhBMP7) is applied for treatment of bone fractures, especially tibial non-unions. Its application may induce autoantibodies (aAB) affecting the targeted and endogenous signaling pathways and in turn negatively impact treatment efficacy.
Methods: Novel and sensitive assays for the quantification of BMP7-aAB and BMP2-aAB were established and used to analyze serum samples from healthy controls (n = 100 men, n = 100 women) and patients with long bone fracture (n = 265) treated or not with rhBMP7. Sera from three to nine time points per patient were available and enabled the evaluation of aAB over a time course of up to one year. Functional activity of the BMP-aAB was tested with a BMP-responsive cell-based reporter assay. Consolidation of the fracture was evaluated as clinical outcome potentially affected by BMP7-aAB.
Results: Prevalence of BMP7-aAB and BMP2-aAB was 1–2.5% in non-treated patients or healthy controls. The rhBMP7 treatment induced a transient increase in BMP7-aAB in a subset of patients, returning to non-detectable levels within six months. IgG from BMP7-aAB positive sera inhibited dose dependently the BMP7-reporter gene activity, whereas control sera were without effect. Successful consolidation of the fracture was observed in the majority of both aAB-positive and aAB-negative patients.
General significance: We conclude that BMP7-aAB can be detected as natural aAB in healthy subjects, and are transiently induced by rhBMP7 therapy in a subset of patients. The aAB are capable of antagonizing BMP7 signaling in vitro, but do not preclude treatment success in patients.

1. Introduction

Bone is a tissue with a remarkable regenerative potential controlled in part by bone itself and by the interplay with the immune and vascular systems [1]. After fracture, bone often regenerates completely to its original composition without the formation of a scar. It can therefore be considered as a truly regenerative tissue. Usually, a fracture gap is closed within 3–6 months after trauma. However, some fractures (approx. 10%) show healing difficulties leading to delayed healing, or non-unions also known as pseudarthrosis. There are a number of parameters affecting the healing process including the severity of the initial insult as well as age and health of the patient [2–4].

A compromised healing situation requires an intervention to reactivate and enhance natural bone formation. The major aspects that need to be considered are osteogenic cells, osteoconductive scaffolds, osteoinductive stimulants (hormones and local growth factors) and the mechanical environment, summarized as the diamond concept [5] which was extended by the aspect of vascularity [6]. Interventions according to the diamond concept involve an assessment of all of these aspects for a given patient and the attempt to optimize the therapeutic measures resulting in an individualized therapy plan. Treatment of non-unions following this concept proved to be a reasonable and successful strategy [7–9]. In early stage the therapeutic treatments in delayed union may include biophysical stimulation, e.g. full weight bearing, low-intensity pulsed ultrasound, shockwave or electromagnetic field stimulation. Biological enhancement of bone regeneration is the base in treatment of non-unions. Autologous cancellous bone graft is considered the gold standard in the surgical treatment of non-unions, but the limited availability is problematic [15]. Amongst the locally

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applied biological enhancers are calcium phosphate or collagen sponges as osteoconductive material, growth factors like erythropoietin, fibroblast growth factors or bone morphogenetic proteins (BMPs) as osteoinductive agents and synthetic polymers or autologous bone as osteogenic material [10].

BMPs belong to the transforming growth factor beta (TGFβ) superfamily and are pleiotropic paracrine growth factors that are involved in the regulation of diverse biological processes such as proliferation, survival, apoptosis, differentiation and migration of cells [11]. The different members of the TFGβ-superfamily perform specific tasks during development and homeostasis in various tissues [12]. The groundwork of BMP research in bone was laid in the late 1960s by Marshal Urist when he showed that implanted demineralized bone induced ectopic bone formation in skeletal muscle [13]. Later, the nature of these bone forming factors were identified and termed BMP [14]. The corresponding DNA was cloned and recombinant protein was expressed shortly after [15].

These achievements paved the way for applying recombinant human BMP (rhBMP) to improve bone regeneration and fracture healing. To this end, especially rhBMP2 and rhBMP7 have been tested and further developed to therapeutic biologicals as osteoinductive growth factors and work most efficient in combination with autologous bone material [16–19]. BMP2 and BMP7 induce osteoblast and chondrocyte differentiation thereby increasing intramembranous and endochondral ossification. Both BMPs have been approved for use in humans by the FDA in 2001 and 2004, respectively, and have shown remarkable therapeutic effects in the last decade. However, concerns regarding their safety and side-effects were raised, especially with respect to ectopic bone formation, osteolysis, induction of autoimmunity, cancer, or problems related to cost effectiveness, collectively limiting their use in recent years [20].

The potential induction of autoantibodies (aAB) against rhBMP (BMP-aAB) may be of clinical relevance for three major reasons. First, BMP-aAB might interfere with the biological activity of the therapeutic rhBMP by neutralizing it. Second, rhBMP-aAB complexes may cause unwanted immune reactions. And third, treatment-induced BMP-aAB might cross-react with endogenous BMP thereby interfering with the regular signaling pathways.

In order to test for natural occurring and treatment-induced BMP-aAB, we developed two novel luminometric assays. We determined the prevalence of BMP7-aAB and BMP2-aAB in healthy subjects and in patients with severe fractures treated or not with rhBMP7, and at time points before and after surgery. Our data indicate that rhBMP7 treatment transiently induces BMP7-aAB in a subset of patients, and that these aAB are antagonists for BMP7 signaling in vitro. However, fracture consolidation was successfully achieved in both BMP7-aAB-positive and BMP7-aAB-negative patients, indicating that these aAB do not preclude treatment success.

2. Material and methods

2.1. Patients

Serum samples from a cohort of 200 anonymized healthy donors (100 males and 100 females, age range; 21 to 40 years) were obtained from a commercial supplier (Invent GmbH, Biotechnology Center Hennigsdorf, Germany). Serum samples from fracture patients were collected at the Department of Orthopedics and Trauma Surgery, Heidelberg University Hospital. Two time points were analyzed from 265 patients with long bone fracture (189 females, 76 males), yielding a total collection of 530 samples. The first time point was around surgical intervention (either pre-surgery or two days after surgery), and the second time point was approximately four weeks after surgery. The patients were categorized into different groups according to whether they have been treated with rhBMP7 or not (Table 1).

Table 1
Study design. Fracture patients were divided into two groups (treated or not with BMP7) that received differential surgical treatments.

BMP7 treatment	Patients [n]	Treatment groups	Patients [n]	BMP7-aAB over time, patients [n]
Fracture treatment w/o BMP7	178	Fresh fractures	145	5
		Pseudarthrosis	33	1
Fracture treatment with BMP7	87	Pseudarthrosis	38	2
		Pseudarthrosis RIA[a] or Spongiosa	21	2
		Pseudarthrosis Masquelet[b]	28	3

[a] RIA, reamer irrigator aspirator.
[b] Masquelet, use of temporary cement spacer.

The study was conducted in accordance with the declaration of Helsinki. All individuals provided written consent to the study protocol. The study was approved by the ethics committee of the Ruprecht- Karls-University of Heidelberg (S-636/2011).

2.2. Quantification of BMP7-aAB and BMP2-aAB

The rhBMP7 used in the osteoinductive therapeutic intervention was used as bait to establish a novel assay for detection and quantification of naturally occurring and therapy-induced aAB against BMP7. For reasons of testing the specificity, a second analogous assay for aAB against BMP2 was established. To this end, 0.1 mg of collagen-free rhBMP7 (Olympus Biotech) or rhBMP2 (Metronic) were labelled with acridiniumester-*N*-hydroxy-succinimid (MACN, InVent Diagnostica GmbH) in an amine-free buffer. The labelling reactions were stopped by adding 1 M Tris, pH 7.5. The MACN-labelled rhBMP7 or rhBMP2 was diluted in buffer (PBS, 1% BSA, 0.1% NaN_3) and separated from unbound MACN using 10 kDa MWCO centrifugal filter units (Centricon Ultracel-10K, Millipore, Eschborn, Germany).

After optimizing the conditions of BMP7- or BMP2-aAB detection and quantification, the following protocol was established and used throughout this study. Serum samples (10 μl per reaction) were incubated with diluted MACN-labelled rhBMP2 or rhBMP7 (100 μl per reaction) and incubated at 4 °C overnight. The next day, IgG were bound by incubation for 1 h shaking at 300 rpm at room temperature with a solution of a 10% protein A slurry in PBS (PorosA®, 50 μl, Applied Biosystems). The samples were washed three times with 1 ml washing buffer (50 mM KH_2PO_4/K_2HPO_4 pH 7.5, 100 mM NaCl, 0.1% TritonX-100), the pellet was precipitated by centrifugation (5 min, 3500 rpm, 20 °C) and the supernatant was aspirated. The chemiluminescence of the bound MACN-labelled rhBMP2 or rhBMP7 was measured in a chain luminometer (Autolumat Plus LB 953, Berthold Technologies, Bad Wildbad, Germany). For the characterization of the assay and as positive controls, anti-BMP7 and anti-BMP2 antibodies were used. Mice were immunized with rhBMP7 and monoclonal anti-BMP7 antibodies were generated by a commercial partner (UNICUS Karlsburg OHG, Greifswald, Germany). A commercial anti-BMP2 antibody (CYT-26591) was purchased (Dianova, Hamburg, Germany). Both antibodies were applied in a concentration of 100 μg/ml in the control experiments.

2.3. Isolation of IgG

Total IgG of BMP7-aAB positive and negative sera were isolated by precipitation with protein A. Serum samples (300 μl) were incubated with a slurry of 50% PorosA® in PBS (600 μl) and incubated overnight at 4 °C under constant agitation. The supernatants were discarded and the pellets were washed six times with PBS. Precipitated IgG were eluted with 25 mM citric acid, pH 2.2. Seven fractions (500 μl each) were