HEALING USING A NONSOLUBLE FORM OF RHBMP-2 RELEASED FROM A FIBRIN MATRIX

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Introduction

This research was designed to investigate a new form of recombinant human bone morphogenetic protein-2 (rhBMP-2) delivered in a fibrin matrix. Generally, bone healing in difficult cases requires both stable fixation and the use of autogenerous cancellous bone grafts (ACBG). However, this requires harvesting of significant amounts of ACBG despite the additional morbidity at the donor site. One alternative therapy could be the use of recombinant osteogenic proteins. However, it is known that a single application of rhBMP-2, one of the most potent factors available, without a carrier does not strongly influence the healing of bone defects due to the rapid clearance from the wound site. Therefore, a biodegradable matrix with a controlled release of the rhBMP-2 is necessary to achieve a strong healing potential. Here, we show the results of use of nonglycosylated BMP-2 in a fibrin matrix both in vitro as well as in systematic in vivo studies in the rat and dog.

Method:

Fibrin gels were made for either in vitro release studies (analyzed by direct ELISA) or in vivo healing studies from purified fibrinogen.

For the in vivo healing studies, two separate models were employed. For the rat critical size cranial defect model, the peristomeum was completely retracted and an 8 mm craniotomy defect was created. The surgical area was flushed with saline to remove bone debris and a preformed fibrin gel was placed within the defect. The animals were randomly divided into six groups (n=6): fibrin alone, fibrin + 1, 5 or 20 µg tgBMP-2, fibrin with 1 µg glycosylated BMP-2 and fibrin with 1µg tgBMP-2 +1.3 µg heparin. Doses of BMP-2 are specified as µg per 100 µL gel. All animals were sacrificed at three weeks postoperation and subsequently analyzed with radiographs and histology.

In 10 consecutive canine patients requiring a pancarpal arthrodesis the ACBG was replaced by a 600 µg/ml n-rhBMP-2/fibrin composite, injected as a liquid in the bone defects at the end of the surgical procedure. The healing of the arthrodesis was evaluated using control radiographs at postoperative week 4, 8 and 12. A healing score had been developed and applied by a independent board certified radiologist (Tbl. 1). The results were compared to a retrospective control group of pancarpal arthrodesis performed using an ACBG.

Tbl 1: Radiographic healing score
0 point no mineralized tissue in the joint gap
1 point mineralized tissue visible in the joint gap
2 points bony bridging of the joint gap
3 points remodeled bone, subchondral bone plate remodeled

Result:

When n-rhBMP-2 was mixed into fibrin matrices and retention was tested in vitro, a significant fraction of the molecule was shown to be retained in the matrix such that when 10 µg/ml was added, 83% was retained. This was significantly higher than other forms that were tested, including glycosylated BMP (27%) and n-rhBMP-2 premixed with heparin (21%). Each of these formulations were then tested in vivo in the rat critical size cranial defect and a direct correlation was observed whereby the retained n-rhBMP-2 showed an extremely strong healing at 3 wk (74%, 92% and 98% for treatment with 1,5 and 20 µg/ml respectively) while treatment with 1 µg/ml of glycosylated BMP-2 or n-rhBMP-2 bound to heparin led to much worse healing (46% and 51% respectively). Healing with fibrin alone was 14%.

Healing of the canine arthrodesis was then explored with the best formulation tested in the rats: n-rhBMP-2 in fibrin. In this prospective study, no dog showed local or systemic effects or had postoperative complications like infection or implant loosening. The level of healing for the n-rhBMP-2 treatment can be seen at all timepoints as compared to ACBM in Fig 1.

Discussion

In vitro studies showed that n-rhBMP-2 is released more slowly from the fibrin gel than other more soluble forms of rhBMP-2 (glycosylated rhBMP-2 or rhBMP-2 mixed with Heparin). In the critical size cranioriomy model in rats the deficits treated with fibrin gel and n-rhBMP-2 healed significantly better than gels with soluble forms of rhBMP-2 which diffuse faster out of the gel, showing that a controlled release over several days is important for a good healing response of the bone. However, clinically, a carpal arthrodesis in the dog is a much more challenging problem. Without an ACBG the bony bridging occurs very slowly, often leading to implant loosening and in many cases failure. But even with an ACBG, only 39% of dogs showed radiographic healing of the arthrodesis after 17-30 weeks. The results of the series treated with n-rhBMP-2 showed healing either as good or better than autograft without formation of any ectopic bone. The formation of mineralized tissue in the joint gaps was visible earlier in all dogs of the n-rhBMP-2 group and the long-term follow up control radiographs and the histology revealed further healing and remodeling of the new formed bone indicating a metabolically active and functional bone. If the n-rhBMP-2 dose is calculated for the defect volume, 0.2-0.8 µg n-rhBMP-2/mm² defect was used in the rats and 0.4-1.2 µg n-rhBMP-2/mm² defect in the dogs. The successful healing was obtained using dosing in the same range for both species. That is a novel and powerful finding, because the regenerative capacity of mammalian bone is believed to be inversely proportional to the position on the phylogenetic scale. This prospective clinical study with pancarpal arthrodesis in the dog demonstrates the effect of using n-rhBMP-2 in a fibrin matrix for the treatment of bone defects and the very strong healing response of the canine bone suggests that n-rhBMP-2 mixed into a specially designed fibrin matrix could provide a clinically beneficial therapy for many human orthopedic applications.