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Use of BMPs and bisphosphonates to improve bone fracture healing

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1. ABSTRACT

In orthopaedics, focus is often placed on increasing bone formation by an anabolic drug like the recombinant human bone morphogenetic protein (rhBMP). However, premature or excessive bone resorption, due to stress-shielding, instability or infection/inflammation can lead to poor, delayed, or absent bone union. Anti-catabolic drugs such as bisphosphonates have therefore been explored to improve bone repair. This short review discusses the current literature underlying the anabolic-catabolic paradigm for bone repair with a focus on BMP and bisphosphonate combination approaches.

2. INTRODUCTION

Recombinant human bone morphogenetic proteins (rhBMPs) are potent stimulators of bone anabolism that are in clinical use for the treatment of non-union and critical sized bone defects. Although BMPs stimulate bone anabolism, they also increase osteoclast differentiation, mature osteoclast survival, and osteoclastic bone resorption (1-3). Furthermore, BMPs can stimulate osteoclastogenesis indirectly through osteoblasts via the RANK/RANKL pathway (4). In a clinical setting, this can lead to the premature resorption of BMP-induced bone if early resorptive stimuli predominate. Several clinical

reports of failure exist because of premature catabolism associated with BMP use. These include loss of fixation in vertebral fractures, premature allograft resorption in the spine (5), and some cases of failure and loosening in hip revision arthroplasty (6).

One approach to prevent premature or excessive bone catabolism associated with BMP application is by pharmaceutical manipulation of the osteoclasts using an anti-resorptive agent (7). Bisphosphonates (BPs) are a class of drugs that bind to bone and inhibit osteoclast-mediated resorption. BPs have been extensively studied experimentally (8-15) and are in clinical use for the treatment of osteoporosis and other metabolic bone disorders. The present review will describe examples to maximise net bone production in an orthopaedic setting by combining BMPs and BPs, aiming for maximal formation and minimal resorption. First we will outline the experimental findings with bisphosphonates and fracture repair that led to such a therapeutic approach and describe a number of instances where systemic BPs have been successfully used in combination with BMPs. Finally, we will summarise the findings of several recent studies focused on combining BMPs with local bisphosphonates.

3. BISPHOSPHONATES AND BONE REPAIR

Bisphosphonates (BPs) are a class of drug that have clinical efficacy for the treatment of osteoporosis and other metabolic bone disorders, and are increasingly being explored for orthopaedic indications. Newer bisphosphonates have increased potency due to the presence a nitrogen-containing side chain (15). Nitrogen-containing bisphosphonates (pamidronate, alendronate, risedronate, ibandronate, zoledronic acid) inhibit an enzyme called farnesyl diphosphate (FFP) synthase which leads to cellular dysfunction and apoptosis, while the older non-N-BPs (clodronate) produce toxic metabolites in the mitochondria (14).

Bisphosphonates accumulate at sites of bone mineral deposition, in particular in regions of high bone turnover such as a fracture site (12). The drug is released during subsequent bone resorption and internalised by osteoclasts leading to inhibition of osteoclast activity and/or osteoclast apoptosis. Consequently, bisphosphonates are potent inhibitors of osteoclast mediated bone resorption (14).

Bone repair is a dynamic and complex process that features an early inflammatory response, followed by formation of a soft callus, its replacement with a hard mineralized callus, and finally remodeling (16). Premature and excessive remodelling of the hard callus might occur also during fracture healing, due to stress-shielding or instability, and might result in a weaker initial union, or even a delayed or non-union. The capacity of bisphosphonates to improve repair by preventing early remodelling have been examined in a number of models.

In a rat open osteotomy model, significant increases in callus volume and bone mineral content were seen with pamidronate (PAM) treatment. This led to a 60% increase in strength compared to controls at 6 weeks post-operatively (11). In a closed fracture model, rats treated with the more potent bisphosphonate zoledronic acid (ZA), again showed increases in callus size and strength (12). Subsequent studies investigating various dosing regimens indicated that a single bolus systemic dose could provide equivalent benefits to bone strength compared to multiple smaller intermittent doses. The single dose, however featured superior bone remodelling following union (17). Benefits were also seen in rabbit models of distraction osteogenesis, an orthopaedic process that is also depending on an adequate anabolic response (8, 10). In a randomised study of patients operated with a tibial osteotomy for gonarthrosis, the time to clinical healing was evaluated (18). The patients were given either ZA 5 mg i.v. or saline four weeks postoperatively as a single infusion. The extraction torque of the screws for the external fixator used to secure the osteotomy during healing was higher in the treated group, however, no effect could be seen by the ZA infusion regarding time to healing.

However, bone repair is primarily an anabolic process and bisphosphonate treatment only has the potential to maximise the effects of a system's intrinsic bone forming potential (6). Thus it was speculated that bisphosphonates could be effectively combined with bone forming agents such as BMPs, which themselves can also stimulate bone resorption (1-4).

4. LOCAL BMP AND SYSTEMIC BISPHOSPHONATE TREATMENT

A simple model to demonstrate synergy between BMPs and BPs is the rat tibial bone chamber model (Figure 1) (19). An allograft is inserted into the chamber and ingrowth can occur from one end of the graft. Using the ingrowth distance into the graft at 6 weeks as anabolic equivalent, an increase was caused by rhBMP-7 compared to saline or ZA alone. Similarly, using the BV/TV as anti-catabolic outcome, a more dense bone was found in the remodelled part of the allograft in the group receiving a single systemic dose of 0.1 mg/kg ZA at 2 weeks, compared to BMP or saline. Combining the two, rhBMP-7 + ZA co-treatment increased both parameters resulting in both increased ingrowth distance and increased density and with an increased net bone formation, compared to all other groups (bone graft only, rhBMP-7, and ZA only) (Figure 2). It was concluded that the unloaded bone chamber constitutes an extremely pro-catabolic environment and that systemic ZA was effective in preventing resorption and optimising the net bone. However, one study has shown less positive effects with local BMP and bisphosphonate icadronate co-delivered via type I collagen carrier in a rat ectopic bone formation model. Gong *et al.* demonstrated adjunct treatment with 5 microg rhBMP-7 and systemic incadronate (1 microg/kg thrice weekly, 3rd-7th weeks post-implantation) to block bone maturation and formation, as well as significantly reducen tartrate resistant acid phosphatase-positive cells numbers (20).

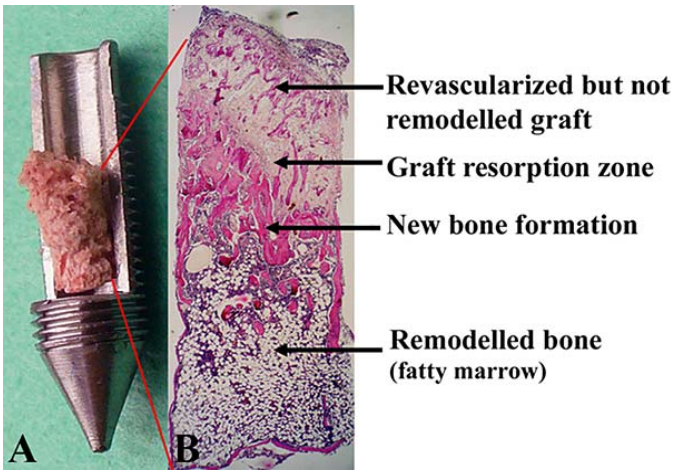

Figure 1. (A) Bone conduction chamber with freeze-dried bone graft, allowing mesenchymal tissue growth from the bottom chamber inlets upward into the bone graft. (B) Histological haematoxylin & eosin stain of untreated graft following 6 weeks insertion. Host-tissue ingrowth is from the bottom of the chamber as the graft is revascularized. In this unloaded model, the newly formed bone almost entirely resorbed and remodelled into fatty cell marrow.

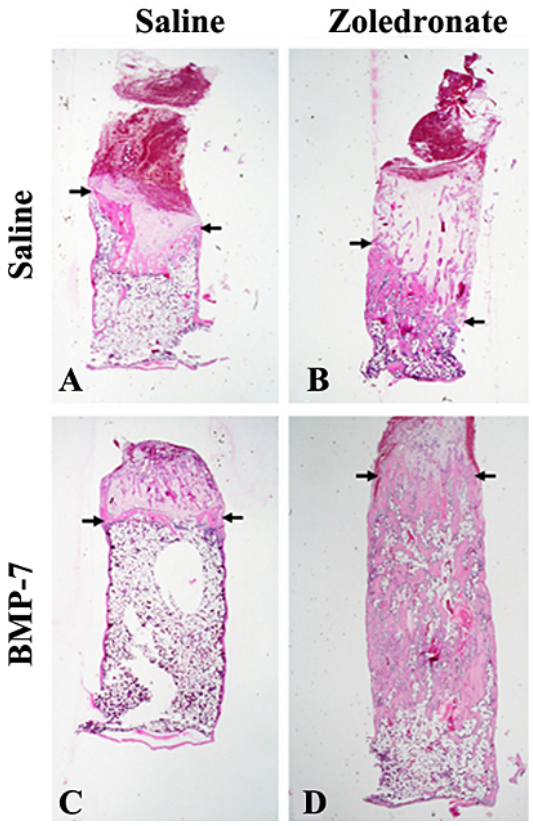

Figure 2. Haematoxylin & eosin stained representative samples showed (A) Untreated control demonstrated remodelled bone graft after 6 weeks insertion in chamber. The bone ingrowth front is indicated by the arrows, and the tissue below has been remodelled. (B) In the zoledronic acid (Zoledronate) treatment group, the remodelled bone below the ingrowth front showed 3-4 times more bone compared to controls. (C) In the BMP-7 treated groups, the bone ingrowth front (arrows) were 50-100% deeper into the bone graft compared to controls. The newly formed bone below the bone ingrowth front had mostly been resorbed and replaced by fatty marrow. (D) rhBMP-7/zoledronic acid co-treatment led to increased ingrowth distance and increased bone retention.

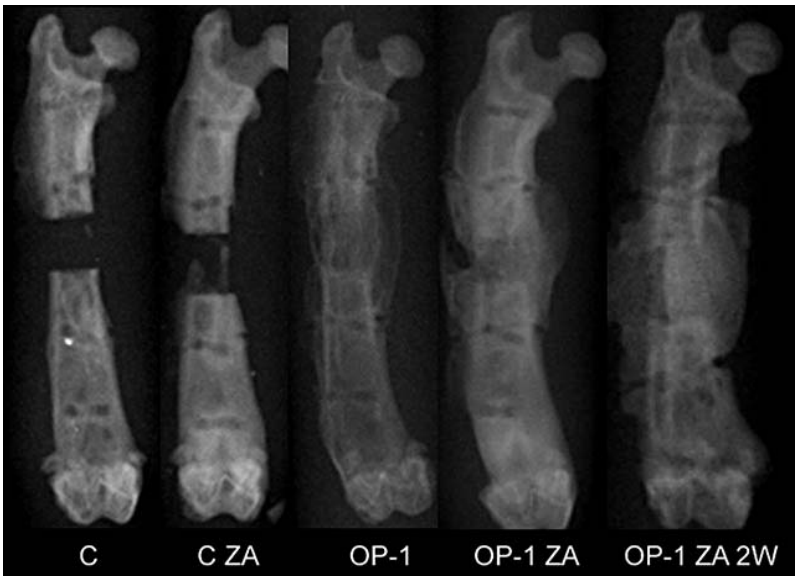

Figure 3. Radiographic X-rays demonstrating increased callus formation with co-treatment of rhBMP-7 (OP-1) and systemic zoledronic acid (ZA). Moreover, delaying systemic co-treatment to 2 weeks post-operatively led to further increased callus formation.

Further convincing evidence for synergy between local BMP and systemic BP reported also in a more clinically relevant model was shown in a rat critical sized defect model (9). This model features a deficient anabolic response that leads to a 100% healing failure. Addition of 50 microg rhBMP-7 (OP-1) to the fracture gap was sufficient to lead to a tenuous union in the majority of animals at 8 weeks. The addition of a single systemic bolus dose of ZA further dramatically increased callus formation leading to an 87% increase in callus volume and an 107% increase in callus strength compared to rhBMP-7 alone. Notably, there was a significant benefit delaying the dosing of systemic ZA to 2 weeks after surgery rather than administer the drug at the time of surgery (Figure 3). By speculation, the early bony callus at 2 weeks may be able to capture and retain additional bisphosphonate compared to a newly fractured bone.

This combination of an anabolic and an anti-catabolic drug may have additional benefit in the context of genetic disease, such as Neurofibromatosis type 1 (NF1) – a condition featuring decreased bone anabolism and an increased potential for catabolism (21). In a study with NF1-deficient mice that showed a reduced response to intramuscular implantation of 20 µg rhBMP-2, co-treatment with systemic ZA (5 doses of 0.02 mg/kg over 3 weeks) was able to maximize the amount of bone produced (22). This approach has been found to be effective in a clinical case series for NF1 tibial pseudarthrosis patients treated with BMP and BP (23), although adjunctive pharmaceutical treatment was also found to depend on effective surgical fixation. Further studies will be needed to demonstrate the value of this strategy for intervening in other genetic bone diseases.

5. LOCAL BMP AND BISPHOSPHONATE CO-TREATMENT

The first report of a combined local application of BMP and BP to a bone graft was in the rat tibial bone chamber (24). Clodronate (CLOD) a first generation BP was administered locally to an impacted bone graft model with non-impacted grafts as controls with or without addition of BMP-7. CLOD was found to increase bone graft density and eliminate the transient bone resorptive effect of BMP-7. However, local CLOD did also reduce the ingrowth distance of new bone into the graft. Adding BMP, the negative effect of both graft impaction as well as CLOD administration could be at least partly reversed (24). Interestingly, this study implied that the anti-anabolic effects of CLOD may be more significant when applied locally. Recently, the same rat bone chamber model was used to explore the combination of locally applied BMP-7 + ZA, added to non-impacted allografts (25). An almost four-fold increase in net formation of bone was found by the combination of BMP and ZA compared to saline. The ingrowth distance of both ZA alone as well as the combination BMP-7 and ZA, however appeared to be less than in the previous series using systemic bisphosphonates, implying an anti-anabolic effect of the bisphosphonate.

In another study, rhBMP-2 and the bisphosphonate minodronate were locally co-delivered in a rat intramuscular ectopic bone formation model. Addition of the bisphosphonate prevented bone loss over 4 weeks and led to a net increase in bone density and mechanical strength over rhBMP-2 alone (26). In a follow-up study, the ectopic bone was successfully used as a graft material in a femoral muscle pedical flap model (27).

Lastly the combination of BMP-2 and a potent bisphosphonate, ibandronate, has been trialled in the piglet model of ischemic necrosis, where collapse of the femoral head occurs routinely (28). In comparison to the control group, the combined therapy group had a significant decrease in femoral head deformity with a significant increase in the trabecular bone volume and osteoblast surface. One cautionary finding was the presence of heterotopic ossification in the hip joint capsule, requiring further refinement of delivery.

Not all local bisphosphonate studies have yielded favourable outcomes. Local delivery of high doses of PAM (2 mg and 3 mg) in a rat calvarial defect model was found to impair bone repair (29). While this model did not feature rhBMP treatment, similar impairment was found in a canine titanium implant fixation model with morselised bone graft soaked in high dose Alendronate (ALN). Furthermore, local ALN-treated grafts showed inhibited new bone formation and reduced resorption of the graft material (30). Later, another canine implant fixation model by the same group also found similar impaired implant fixation with 450 microg rhBMP-2 and bone allograft were treated with 9 mg/ml PAM (31). Notably, all of these studies featured a high local concentration of bisphosphonate, and as reviewed by Schindeler and Little, such doses have been reported to be cytotoxic based on *in vitro* studies (13).

These observations led us to hypothesise that the capacity of local bisphosphonates to positively modulate the formation and retention of rhBMP-induced bone would be dose dependent. This concept was confirmed in recent study in a mouse intramuscular ectopic bone formation model (32). Variable doses of the bisphosphonate PAM were combined with a constant 25 microg dose of rhBMP-7 in a solvent-cast poly-D,L-lactic acid (PDLLA) polymer delivery system. It was found that high 2 mg local dose of PAM dramatically impaired the formation of rhBMP-7 induced bone. In contrast, lower dose of 0.02 mg PAM was able to yield an overall increase in net bone. Similarly, in a follow up study to their bone grafting work, Jakobsen *et al.* also showed that the effects of local zoledronic acid differed between a low dose where a favourable increase of bone in the gap and on the implant surface was seen, whereas higher doses were inhibitory. These data illustrate the importance of appropriate bisphosphonate dose selection for pre-clinical and clinical applications (30).

6. CONCLUSIONS

There is a growing body of pre-clinical evidence, particularly in rodent models, to support the biology behind BMP and bisphosphonate as dual interventions. In this review we have discussed the progression from use of bisphosphonates alone in systems featuring sufficient anabolism to facilitate bone healing to combinations of BMPs with systemic and local bisphosphonates. One continually emerging theme is that bisphosphonate dosage, particularly when applied locally, is critically important for the eventual outcome.

The number of studies specifically examining synergy between BMPs and bisphosphonates are limited. More research, particularly focused on timing of systemic doses, dosing rates, as well as local co-delivery systems for both agents will be required for translation to clinical practice. The combination of other anabolic and anti-catabolic agents is also being actively explored.

7. ACKNOWLEDGEMENTS

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