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Investigating the synergistic efficacy of BMP-7 and zoledronate on bone allografts using an open rat osteotomy model

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ABSTRACT

Bone grafts are well-established in the treatment of fracture non-unions but union is still not always achieved. Harvesting autograft is associated with donor site morbidity and the available amount of bone is limited. Allograft is more easily obtained and available in greater quantities but lacks the osteoinductive characteristics of autograft. We have previously shown a synergistic effect of bone morphogenetic protein (BMP-7), systemic bisphosphonates and autograft. In the present study we hypothesized that the combination of allograft + BMP-7 + systemic ZA is more effective than autograft alone, which is currently the most frequently used aid in augmenting fracture and non-union healing.

Femoral osteotomies were performed on 82 male Sprague Dawley rats and fixed with intramedullary K-wires. The rats were randomized into 7 groups: i. saline, ii. autograft, iii. allograft, iv. allograft + BMP-7, v. autograft + zoledronate (ZA), vi. allograft + ZA and vii. allograft + BMP-7 + ZA. Autografts were harvested from the contralateral tibia. Allografts were obtained from donor rats and frozen. BMP-7 was administered locally in the form of a putty placed circumferentially around the osteotomy. At 2 weeks, the rats were injected with a single dose of either saline or ZA. The rats were sacrificed at 6 weeks and the femurs were evaluated using radiography, histology, μCT and three-point bending tests.

Complete radiological healing was seen in all rats in the BMP-7 groups. The callus volume was larger and the calluses were denser with allograft + BMP-7 + ZA than in all other groups (μCT, $p < 0.001$). Mechanical testing yielded a substantially higher peak force with the allograft + BMP-7 + ZA combination than all other groups ($p < 0.01$, $p < 0.001$). This was further reinforced in the 59% increase in the peak force observed in the osteotomized femurs of the allograft + BMP-7 + ZA group compared to the control femurs ($p < 0.01$), whereas significant decreases of 22–27% were observed in the saline or bone-graft alone groups ($p < 0.01$, $p < 0.05$). Thus our results suggest that allograft combined with the anabolic effect of BMP-7 and the anti-catabolic effect of zoledronate is more efficient than autograft alone.

Introduction

Successful fracture repair is an optimal biological response by which new bone is regenerated, remodeled and pre-injury skeletal function is regained. Repair comprises a complex series of coordinated processes regulated by signaling pathways that elicit phased anabolic and catabolic responses. An anabolic response commences with the initial acute inflammatory stage of bone repair and is characterized by the formation of new bone by osteoblasts leading to the gradual bridging of the fracture site and restoration of mechanical integrity. A catabolic response is also initiated at the time of fracture, wherein dead or fractured bone is resorbed and subsequent new bone is remodeled by osteoclasts. Both the anabolic and catabolic phases are intrinsically coupled and balanced responses. Occasionally, complications such as delayed fracture unions or non-unions can be attributed to a disruption of this balanced anabolic–catabolic paradigm. In such instances, biological, mechanical and pharmacological manipulation of these anabolic and catabolic processes may enhance the fracture healing [1–4].

Use of bone grafts is well-established in the treatment of fracture non-unions [5–8]. Autologous bone graft treatment remains the gold standard in clinical practice as it offers an optimal combination of an osteoconductive scaffold, osteoinductive stimulants and osteogenic cells. Limitations, however, exist in harvesting autograft with prolonged theater time, morbidity associated at the donor site, its restricted availability and viability [5,9]. Allograft is an alternative grafting material that is more easily obtained and is available in much greater quantities. But the processing and sterilization required of allograft render it an essentially necrotic material, significantly

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weakening its clinical bone forming potency [10,11]. Consequently, even with the use of either autograft or allograft in treating fracture non-unions, union may still not always be achieved [5,12].

Bone morphogenic proteins (BMPs) can be used to either replace the bone graft or be added to the bone graft to augment the osteogenic drive. BMPs are growth factors influencing cascades of pathways involved in skeletal development, regeneration and homeostasis [13–15]. In the half century since the first observation of the osteoinductive potential of BMPs by Urist [16], their molecular structures, mechanisms of action and efficacy in enhancing bone repair have been extensively examined and elucidated [13,17–19]. Data from animal models of critical-sized defects have shown the results of BMP-induced bone regeneration to be at least equivalent to autologous bone graft, with two recombinant forms, rhBMP-2 and rhBMP-7, currently approved for clinical use [13,17,19]. Although regarded as potent stimulators of bone anabolism, the potential for BMPs to stimulate osteoclastogenesis and induce premature bone/callus resorption has also been documented given the intrinsic coupling mechanisms that exist between osteoblasts and osteoclasts [13,18,20,21]. While the literature frequently attributes the RANKL/RANK pathway as the molecular mechanism by which this occurs [20–22], other studies have postulated the existence of a more complex interplay at work [18,23–25]. Thus, while the molecular modes of action of BMPs is yet to be fully elucidated, in order to optimize the use of BMPs it is important to uncouple its effects from bone resorption.

This avenue of investigation has been pursued by the use of an established class of drugs, bisphosphonates, which are primarily of interest for their anti-catabolic effects in modulating BMP-induced osteoclastic resorption [23,26–31]. Bisphosphonates demonstrate a high selectivity for – and adsorption to – mineral surfaces in bone, particularly regions undergoing high bone turnover, including fracture sites [26,32–34]. Subsequently, they are internalized by osteoclasts leading to disruption of specific metabolic reactions and consequent osteoclast dysfunction and apoptosis [26,33]. Specifically, the current generation of nitrogen-containing bisphosphonates function by inhibiting several key enzymes of the mevalonate pathway, with farnesyl pyrophosphate synthase identified as their primary target [26,35]. Bisphosphonate therapy is established in improving the clinical outcomes of osteoporosis and several metabolic bone disorders characterized by excessive bone resorption [26,27,36]. Moreover, its potency in preventing premature resorption of newly-formed bone has also been demonstrated in animal models of critical sized defects through the use of optimally-timed dosages of bisphosphonates [23,28,30,31,37–39].

We have previously shown a synergistic effect of systemic bisphosphonates (specifically zoledronate, ZA) together with BMP-7 and fresh autograft [40]. Using an open rat femur osteotomy model, the combination of autograft and BMP-7 yielded a doubling of the callus size and strength relative to autograft alone at six weeks. However, a diminished bone volume fraction was also noted and can, by speculation, be attributed to the remodeling/resorptive influence of BMP-7. This influence could be reversed by the administration of a single, systemic dose of zoledronate at two weeks, resulting in an increased callus size, bone mineral content and a further doubling of the strength compared with the combined autograft and BMP-7 treatment alone. In sight of the limited availability and donor-site morbidity associated with autograft, we also demonstrated the synergistic potential of frozen allograft, BMP-7 and zoledronate in a controlled environment (i.e. a bone conduction chamber) in rats [41]. The treatment of allograft with both BMP-7 and zoledronate dramatically augmented the mineral content by more than a factor of 4 compared to the saline treated control allograft.

As a natural progression, in this study our aim is to extend this line of investigation to assess the potential of combinations of allograft, BMP-7 and zoledronate in enhancing fracture healing. Thus, we revisited the effectiveness of fresh autograft versus frozen allograft and we hypothesized that allograft + BMP-7 is superior to autograft, when combined with systemic zoledronate treatment in an open rat osteotomy model.

Methods

Experimental model

The experimental model in this study is an open fracture model of recalcitrant non-unions previously described by Tägil et al. [42] and is in contrast to the more commonly used closed fracture model [43]. With the open fracture model, approximately 50% of the fractures result in non-unions at the six-week time point. It is unknown if non-unions at six weeks are indicative of failure to unite further along in time or simply delayed healing. In the closed fracture model all fractures are healed at the same time point. Thus, the six-week time point is solely a reference point at which comparisons can be made as to the efficacy of the treatments under consideration.

The femoral osteotomies were performed on 82 male Sprague Dawley rats (60 days old; 300 g mean weight; Taconic M&B A/S, Ry, Denmark). The rats were randomized into 7 different groups: i. saline, ii. autograft, iii. allograft, iv. allograft + BMP-7, v. autograft + ZA, vi. allograft + ZA and vii. allograft + BMP-7 + ZA. The rats were housed in pairs, permitted unrestricted weight bearing and had ad libitum access to food and water. A one week acclimatization period was allowed prior to surgeries. Care and experimental protocol was approved by the local animal ethics and scientific advisory committee (Ethical Permission No. M216-08).

Surgery and drug administration

Anesthesia was induced with a solution containing saline, diazepam (2.5 mg/mL) and pentobarbitalnatrium (15 mg/mL) administered intraperitoneally. Antibiotic prophylaxis in the form of streptocillin was given preoperatively. The osteotomy was made in the left femur of each rat. Under standard sterile conditions, a lateral approach was made and the periosteum was circumferentially incised. A transverse osteotomy was performed at the mid-diaphysis using an oscillating power saw. The fracture site was stabilized and fixed with an intramedullary K-wire. Based on the randomization group, the corresponding treatment was administered. Fresh autologous bone graft was harvested from the contralateral proximal tibia via an anteromedial incision and the use of a 2.7 mm drill just distal to the physis. Allografts were obtained from rats of same origin and were frozen in liquid nitrogen before being morselized in a mortar. BMP-7 (Osigraft, Stryker Biotech, Malmö, Sweden) was administered locally in doses of 50 μg corresponding to the maximum recommended dose in humans [2,23,44]. BMP-7 was prepared in the form of a putty consisting of 2 mg of BMP-7 per 570 mg bovine collagen admixed with 200 mg carboxymethylcellulose (CMC). The putty was subsequently mixed with bone graft and placed circumferentially around the osteotomy. Immediately following surgery and on subsequent days, the rats received analgesic (buprenorphine subcutaneously and paracetamol). At 2 weeks post-surgery, the rats received a single subcutaneous injection of either saline or ZA (0.1 mg/kg) (Zometa, Novartis, North Ryde, NSW, Australia). The rats were sacrificed at 6 weeks by means of an intraperitoneal injection of pentobarbital sodium. Thereafter, both the intact control and the osteotomized femurs were explanted and all K-wires extracted. All samples were wrapped in saline-soaked gauze and stored at −20 °C.

Evaluation methods

Bone samples were subsequently evaluated using radiographic analysis, histology, μCT and three-point bending mechanical tests.