Autologous bone grafting on steroids: preliminary clinical results. A novel treatment for nonunions and segmental bone defects

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Abstract Clinical management of delayed healing or nonunion of long bone fractures and segmental bone defects poses a substantial orthopaedic challenge. Surgical advances and bone tissue engineering are providing new avenues to stimulate bone growth in cases of bone loss and nonunion. The reamer-irrigator-aspirator (RIA) device allows surgeons to aspirate the medullary contents of long bones and use the progenitor-rich “flow-through” fraction in autologous bone grafting. Dexamethasone (DEX) is a synthetic steroid that has been shown to induce osteoblastic differentiation. A series of 13 patients treated with RIA bone grafting enhanced with DEX for nonunion or segmental defect was examined retrospectively to assess the quality of bony union and clinical outcomes. Despite the initial poor prognoses, promising results were achieved using this technique; and given the complexity of these

cases the observed success is of great value and warrants controlled study into both standardisation of the procedure and concentration of the grafting material.

Introduction

Clinical management of delayed healing or nonunion of long bone fractures and segmental bone defects poses a substantial orthopaedic challenge. The optimal treatment must address both the biological and mechanical requirements, and traditional solutions include transfer of vascularised bone, distraction osteogenesis and the use of cancellous autografts/allografts [1]. On the other hand, a growing armamentarium of novel biological and tissue engineering approaches offer orthopaedic surgeons additional strategies to enhance bone healing in difficult clinical situations. Bone tissue engineering is dependent on selection of appropriate cells (differentiated or progenitor cells), osteogenic stimulation, such as the provision of morphogens introduced in the form of recombinant proteins or via gene transfer, and use of biocompatible and mechanically suitable scaffolds for cell delivery [2].

Adult mesenchymal stem cells (MSCs) play a pivotal role in cell-based strategies for bone regeneration since they can be easily isolated, expanded, and under appropriate conditions, differentiated into mesenchymal tissues such as cartilage, bone, muscle, fat or marrow stroma [3]. Various proteins have been discovered to participate in controlling the progression from undifferentiated progenitor cell to a lineage-committed osteoblast, including the Wnts, members of the transforming growth factor- β superfamily, Notch, Hedgehog and fibroblast growth factors (FGFs) [4]. Currently, only human recombinant bone morphogenetic protein (BMP)-2 and BMP-7 have been approved for

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clinical use, and their application is restricted to spinal fusion, tibial fracture nonunion and alveolar reconstruction [5, 6]. While studies have demonstrated the efficacy of recombinant human BMP application for spinal fusion, the debate over efficacy for the healing of long bones and the general cost-effectiveness and safety of treatment with BMPs is still ongoing.

Dexamethasone (DEX) is a synthetic steroid that has been traditionally used experimentally to differentiate MSCs into osteoblasts in cell culture, and it has been reported that human MSCs readily undergo osteoblastic differentiation and deposit mineral when exposed to 10^{-7} M DEX, ascorbic acid and β -glycerol for 21 days in vitro [7]. Although effective, this approach is not practical for clinical application since it requires prolonged exposure to DEX. In previous work we showed that short-term in vitro exposure to higher doses of DEX is sufficient to commit MSCs irreversibly to osteoblastic differentiation and is therefore an attractive option to be used intraoperatively during a single surgical procedure in situations where bone restoration is needed [8].

The reamer-irrigator-aspirator (RIA) (Synthes Inc., Paoli, PA, USA) was developed as an alternative method for long bone canal reaming in the process of intramedullary nailing. With the advent of the RIA device, temperature and pressure can be controlled via the simultaneous irrigation and aspiration system built into the reamer. Particles aspirated by the RIA are caught in a coarse filter, from which they can be recovered and used as source of autologous bone graft with osteogenic potential. The “flow-through” fraction of RIA aspirate is rich with progenitor cells and soluble osteogenic factors and can be also used for enhancement of bone regeneration. Recent investigations have confirmed the ability of RIA bone graft harvest to foster bone healing in sheep models [9] and in humans [10–12]. Recent laboratory analyses have also verified the osteogenic potential of the flow-through fraction of RIA aspirate recovered from patients undergoing hip arthroplasty [13].

Here we present a novel, intraoperative, multi-step procedure that uses the RIA system to recover autologous bone along with progenitor cells from bone marrow. The recovered materials are treated with a high concentration of DEX and then implanted into the fracture site. The purpose of this study was to evaluate our preliminary results with this technique.

Materials and methods

Surgical procedure

The surgical protocol was pre-approved by the local Institutional Review Board. The RIA harvest site was typically the contralateral femur of the injured leg, or the

femur ipsilateral to the injured arm. An incision was made at the greater trochanter, and a cannulated drill was used to open the proximal femur, as if a trochanteric femoral nail were to be implanted. This opening was often over-drilled by 2–3 mm to ensure quick and easy access. A terminally beaded guide rod was fed down into the medullary canal through the opening and proper position was confirmed with intraoperative imaging. The canal was then reamed by several passes of the RIA reamer (Fig. 1a). The reamer head size was determined by radiographic measurement of the canal. The size was chosen to take the inner millimetre of the canal at the isthmus. For irrigation, sterile saline was drawn from an intravenous bag via the tube assembly manifold. Solid osseous particles were recovered from the reaming aspirate with an in-line Biomet Redi-Flow® open-pore filter (Fig. 1b) and the filtrate collected in a second sterile vessel (Fig. 1c). While the grafting mixture was being prepared, the reduction and fixation portions of the operation were carried out allowing sufficient infusion time for the grafting mixture. Once graft harvest was complete the wound was irrigated and closed as normal.

Preparation of enhanced RIA grafting mixture for implantation

While the incision and trochanteric opening were being copiously irrigated and closed, the aspirated liquid and bone particles were mixed together and the entire combination was mixed with dexamethasone sodium phosphate (Decadron, Merck & Co., Inc., Whitehouse Station, NJ, USA). The amount of added DEX was calculated in respect to a fluid volume to try to achieve a concentration of 10–5 M (e.g. 1 mg of DEX was added to a 200 ml of liquid material). In three patients, commercially available rhBMP-2 (INFUSE® Bone Graft, Medtronic, Minneapolis, MN, USA) was added to the mixture. This mixture was allowed to incubate until the grafting site was adequately reduced and fixed; this usually took 90–120 min. A gelatin scaffold (Gelfoam, Pharmacia and Upjohn, Kalamazoo, MI, USA) was then used to absorb the mixture (Fig. 2a) and was then carefully packed into the nonunion site or segmental defect (Fig. 2b). Finally, the autologous bone particles were packed around the scaffold with enhanced RIA mixture (Fig. 2c).

Patients

After obtaining informed consent, ten men and six women aged 30–80 (mean age: 52 years) received RIA bone grafting treatment for nonunion or segmental defect between 2006 and 2009. Patients were selected via an automated query of our ORTHO D.U.D.E. database, which compiles categorical case information on all patients treated

Fig. 1 Collection of autologous osseous particles and bone marrow containing mesenchymal progenitors. **a** Medullary canal reaming during the hip arthroplasty procedure. **b** Solid osseous particles recovered with Biomet Redi-Flow® open-pore filter. **c** Liquid fraction within the sterile container containing mesenchymal progenitors

by the Orthopaedic Service. Inclusion criteria were set as any patient receiving RIA bone grafting for nonunion or segmental diaphyseal defect. Exclusion criteria were defined as any patient having less than three months of follow-up or any patient not receiving autologous grafting from the RIA procedure. After exclusions, 13 subjects remained in the study. All patients treated received RIA grafting + DEX, and three of the patients also received

Fig. 2 Transplantation of an enhanced construct into the defect. **a** Gelatine scaffold used to absorb bone marrow liquid filtrate and appropriate dose of DEX. **b** Infused scaffold carefully packed into nonunion site. **c** Autologous osseous particles packed around the scaffold with enhanced RIA mixture

rhBMP-2 with the RIA-DEX mixture. The median time between nonunion surgery and last follow-up was six months, and mean time was eight months. Patient histories and demographics are shown in Table 1.

X-ray analysis

Medical records were analysed for pertinent medical, surgical and demographic data, and all radiographs from date of injury to latest follow-up were assessed. The most recent radiographs were assessed for evidence of definitive healing. Three independent observers (two orthopaedic surgeons and a non-physician researcher) evaluated the most recent radiographs and classified healing progress using a modified Lane and Sandhu scoring system, described in Table 2 [14, 15]. The mean radiographic score was calculated for each patient. In addition, the most recent radiologist reports were reviewed as an independent evaluation of healing. These reports were compared with Lane and Sandhu scores for congruency. The most recent surgeon visit notes were reviewed for clinical indicators of healing. Factors known to impair healing were also identified and considered, including smoking history, diabetes and infections requiring surgical intervention.

Results

Radiographic healing analysis demonstrated encouraging overall results, with the mean total score being 6.28 of 10 possible points. Two patients did exceptionally well, with mean total scores of 10 and 9, and two patients did rather poorly, with mean total scores of 3 and 4.3. The rest of the group did moderately well, with mean total scores between 5.3 and 7.3. All patient scores are shown in Table 1. The patients who did poorly both had grade III open tibial shaft fractures, with patient 5 sustaining severe bilateral crushing trauma and patient 8 sustaining a severe open tibial shaft fracture in a motor vehicle accident. Neither patient was a smoker or diabetic, or had any known substance abuse problems. Neither patient had rhBMP-2 infusion in their procedure, and both had fasciocutaneous or gastrocnemius flaps for massive soft tissue damage at the time of injury. Patient 8 had infection complications requiring subsequent surgical intervention. DEX is known to raise the risk of infection. Our best and worst cases are shown in Fig. 3 and Fig. 4, respectively.

Ironically, the patient who scored a mean perfect '10' on the healing evaluations has a 50 pack-year history of smoking and as of the last clinic visit still smoked. Both of the highest-scoring patients had open fractures, and one (patient 5) received rhBMP-2 as well as DEX in the grafting procedure. Neither patient had any postoperative complications. Age was not found to be a significant factor determining healing in this series, nor was any significant difference found between men and women. Clinically, our results were largely positive, although not necessarily congruent with the radiographic healing scores. The Lane

and Sandhu score was developed as a method of assessing bony union in segmental defects and does not have parameters to address qualitative factors such as pain and walking ability. Our findings demonstrated that some patients can ultimately have good mobility and minimal pain, despite having an imperfect union radiographically.

The average length of follow-up after nonunion surgery was 8.2 months. Patients 6 and 13 had fairly low healing scores, but went on to full union and positive clinical outcome. All patients displayed at least partial union at most recent follow-up; however, only six patients went on to complete union and were discharged from scheduled follow-up. All patients were able to walk, with patients 8, 9, 12 and 13 walking without any assistive devices. Patients 3, 10 and 13 were prescribed a bone stimulator (Forteo®, Lilly, Indianapolis, IN, USA), with clinical results of probable union, incomplete union and full union, respectively. Patient 11 had a broken blade plate at last follow-up, indicative of persistent nonunion despite abundant callus formation. The patient will probably require further nonunion surgery.

Discussion

The RIA device has proven to be demonstrably effective, both in terms of its ease of use and efficacy in harvesting autologous bone material [9–13]. With the RIA device, osteogenic material can safely be harvested from the medullary canal of long bones with relative ease and with less of a physical burden to the patient. The osteogenic viability of this intramedullary aspirate has been verified in vitro and previous investigators have demonstrated that continuous infusion with DEX will readily induce osteoblastic differentiation and subsequent mineral deposition in cultured cells [7, 13].

Earlier methods of reaming were associated with pulmonary complications resulting from embolisation of fat and medullary elements into the venous system [16–18], as well as necrotic bone from the highly elevated temperature and pressure created by conventional reaming methods. Other methods of autologous bone harvest, such as the iliac crest procedure, are fraught with potential complications and long-term postoperative pain [19–21].

Given the impracticality of continuous DEX infusion for surgical applications, a method was developed and tested for a short-term "blast" of DEX that would have the same effect as the long-term infusion [8]. Our previous in vitro work showed that short-term exposure of human bone marrow-derived MSCs to DEX increased alkaline phosphatase (ALP) activity and mineral deposition in a dose-dependent manner. A single, two hour exposure to 10^{-5} M DEX provoked as much ALP at day ten and mineral

Table 1 Overview of the patient histories, demographics and healing scores

Patient	Age/sex	Risk factors	MOI	Fracture type (AO/OTA)	Open/closed	BMP	Time to graft after injury (months)	Length of follow-up (months), clinical comment	Mean healing score	Ambulatory status
1	61/M	Smoker, EtOH abuse	Bilateral crushing trauma	42A	Open, grade II		3	7, incomplete union	6.7	With assistance
2	61/F	Smoker	Fall	12A	Open, grade I		3.5	12, union	10.0	n.a.
3	65/M	2 prior nonunion repair attempts	Fall	32B	Closed	Yes	108	16, probable union	5.3	With cane
4	56/M	None	Bilateral crushing trauma	42B	Open, grade III C		5	7, incomplete union	4.3	With cane
5	48/M	EtOH abuse	Motorcycle crash	33C	Open, grade III A	Yes	8	11.5, union	9.0	With cane
6	55/M	None	Ski accident	11A	Closed		21	7, union	5.7	n.a.
7	46/F	None	Motor vehicle crash	42B	Open, grade III A		18.5	4, incomplete union	3.0	Without assistance
8	44/F	Charcot-Marie-Tooth disease	Charcot-Marie-Tooth disease	Non-traumatic	Closed	Yes	n.a.	9.2, incomplete union	5.7	Without assistance
9	77/F	Osteoporosis	Unclear, remote	12A	Closed		Unknown	5.8, union	7.3	n.a.
10	58/M	Chronic health issues; morbid obesity	Motorcycle crash	33A	Closed		23.2	5, incomplete union	7.3	With 2 canes
11	79/M	Diabetic	Unknown	31A	Closed		10.5	11, persistent nonunion	6.3	With cane
12	67/M	None	Fall	43C	Open, grade II		23.2	6, union	6.0	Without assistance
13	62/F	None	Equestrian accident	Tibial shaft	Open, grade unknown		60	5.5, union	5.0	Without assistance

Mean healing score according to the modified Lane and Sandhu system as shown in Table 2

MOI mechanism of injury, BMP bone morphogenetic protein

Table 2 Modified Lane and Sandhu [14] scoring system used to evaluate pre- and postoperative radiographs

Parameter	Score
Bone formation	
No evidence of bone formation	0
Bone formation occupying 25% of defect	1
Bone formation occupying 50% of defect	2
Bone formation occupying 75% of defect	3
Full gap bone formation	4
Union (proximal and distal evaluated separately)	
Nonunion	0
Possible union	1
Radiographic union	2
Remodelling	
No evidence of remodelling	0
Remodelling of medullary canal	1
Full remodelling of cortex	2
Total points possible per category	
Bone formation	4
Proximal union	2
Distal union	2
Remodelling	2
Maximum score	10

deposition (measured by amount of calcium in cultures) at day 21 as the standard continuous incubation with 10^{-7} M DEX, both with primary and passaged cell cultures derived from RIA aspirate. Based on these excellent results, DEX was used clinically to enhance RIA grafting mixtures in difficult nonunions to further promote bone growth.

Fig. 3 This was chosen by the authors as an example of a very successful case. **a** Preoperative radiograph. The patient had a long-standing left proximal humerus nonunion, 21 months after a skiing injury. The radiograph was taken just prior to nonunion surgery. Of note, the patient suffered an anoxic brain injury during his original fixation procedure, and this has caused him considerable neurological distress in the time since. He is listed as patient 6 in Table 1. **b** **Fig. 4** This patient was chosen as an example of poor post-graft outcome. **a** She suffered an open grade IIIa comminuted right tibia fracture and fibular fracture, as well as a right ankle fracture dislocation in a motor vehicle accident. This radiograph was taken 18.5 months post-injury. She underwent multiple irrigation and débridement procedures, suffered deep osteomyelitis and necrotic bone at the nonunion site, and had full hardware removal plus exchange nailing all prior to this radiograph. After RIA grafting she underwent another irrigation and débridement procedure and split-thickness skin grafting for wound breakdown. She is listed as patient 7 in Table 1. **b** This radiograph was taken 7 months post-graft. Note there is minimal interval healing compared to her pre-graft films. Again seen are the broken screws consistent with auto-dynamisation. Clinically, the patient was walking without assistance at her last follow-up and had only mild pain manageable without narcotics. She was assessed as slowly progressing towards union, again with some very slight consolidation at the nonunion site. Of note, the patient has a history of significant obesity

Immediate postoperative radiograph following the RIA procedure. **c** This radiograph was taken 13 months post-graft. There is abundant callus formation in the fracture gap and clinically this patient was assessed as healed at his most recent follow-up, just before publication of this paper. He had some reluctance to use his left arm, but this was believed to be related to his neurological problems and not the structural integrity of his arm

In addition to our promising laboratory work, there is some clinical evidence that DEX is an effective adjuvant to bone healing. Corticosteroid injection into unicameral bone cysts has been a standard treatment for many years and has been demonstrated to promote bony healing of the lesion [22]. Patients with cranial injuries often receive high doses of DEX. They also frequently form heterotopic bone [23]. Whether DEX administration contributes to this excess bone formation is not known, but this is a subject of clinical interest.

It is important to highlight the difficulty of the individual cases in this series. All patients treated had persisting nonunions or significant segmental defects that failed to heal with standard procedures. Several of the patients had severe open lower extremity trauma that required multiple vascular and skin grafting procedures to repair tissue destruction. In two cases a previous contralateral below-the-knee amputation was performed due to the severity of the injuries. One non-traumatic case involved Charcot-Marie-Tooth syndrome, and two cases were long-standing nonunions with an unclear remote history. Despite the initial poor prognoses, promising results were achieved using autologous intramedullary bone matter as graft, with DEX as the signal molecule. Patients 3, 5 and 9 received rhBMP-2 with DEX in their grafting procedure, although no significant differences were observed in outcomes. Further controlled study would be necessary to elucidate any synergistic effects.

The obvious limitation of this study is that it is really a collection of case reports, and every procedure was unique. Surgical technique and time frame varied between surgeons, and even changed with the same surgeon across different cases. Given the complexity and poor prognoses of these cases, however, the observed success is of great value and warrants controlled study into both standardisation of the procedure and concentration of the grafting material.

References

1. Biasibetti A, Aloj D, Di Gregorio G, Massè A, Salomone C (2005) Mechanical and biological treatment of long bone nonunions. *Injury* 36(Suppl 4):S45–S50
2. Siddappa R, Fernandes H, Liu J, van Blitterswijk C, de Boer J (2007) The response of human mesenchymal stem cells to osteogenic signals and its impact on bone tissue engineering. *Curr Stem Cell Res Ther* 2:209–220
3. Caplan AI (2009) Why are MSCs therapeutic? New data: new insight. *J Pathol* 217:318–324
4. Deng ZL, Sharff KA, Tang N, Song WX, Luo J, Luo X, Chen J, Bennett E, Reid R, Manning D et al (2008) Regulation of osteogenic differentiation during skeletal development. *Front Biosci* 13:2001–2021
5. White AP, Vaccaro AR, Hall JA, Whang PG, Friel BC, McKee MD (2007) Clinical applications of BMP-7/OP-1 in fractures, nonunions and spinal fusion. *Int Orthop* 31:735–741
6. Jones AL, Bucholz RW, Bosse MJ, Mirza SK, Lyon TR, Webb LX, Pollak AN, Golden JD, Valentin-Opran A et al (2006) Recombinant human BMP-2 and allograft compared with autogenous bone graft for reconstruction of diaphyseal tibial fractures with cortical defects. A randomized, controlled trial. *J Bone Joint Surg Am* 88:1431–1441
7. Diefenderfer DL, Osyczka AM, Garino JP, Leboy PS (2003) Regulation of BMP-induced transcription in cultured human bone marrow stromal cells. *J Bone Joint Surg Am* 85-A(Suppl 3):19–28
8. Ivkovic A, Porter RM, Glatt V, Liu F, Vrahas MS, Harris MB, Estok DM 2nd, Evans CH (2008) Short term exposure to dexamethasone significantly enhances osteoblastic differentiation of human mesenchymal progenitors. Poster presentation, 2008 meeting of the Orthopedic Research Society (ORS)
9. Hammer TO, Wieling R, Green JM et al (2007) Effect of re-implanted particles from intramedullary reaming on mechanical properties and callus formation. A laboratory study. *J Bone Joint Surg Br* 89:1534–1538
10. Scharfenberger A, Weber T (2005) RIA for bone graft harvest: applications for grafting large segmental defects in the tibia and femur. Poster presented at the Orthopaedic Trauma Association 21st Annual Meeting, Ottawa, Ontario, Canada
11. Rudd J, Norris BL, Stafford PR et al (2007) Reamer irrigator aspirator (RIA) bone graft harvesting in non-union and segmental defect repair. Poster presented at the American Academy of Orthopaedic Surgeons Annual Meeting, San Diego, CA
12. Stafford PR, Norris B (2007) Reamer-irrigator-aspirator as a bone graft harvester. *Tech Foot Ankle Surg* 6:100–107
13. Porter RM, Liu F, Pilapil C, Betz OB, Vrahas MS, Harris MB, Evans CH (2009) Osteogenic potential of reamer irrigator aspirator (RIA) aspirate collected from patients undergoing hip arthroplasty. *J Orthop Res* 27:42–49
14. Lane JM, Sandhu HS (1987) Current approaches to experimental bone grafting. *Orthop Clin North Am* 18:213–225
15. Karaoglu S, Baktir A, Kabak S, Arasi H (2002) Experimental repair of segmental bone defects in rabbits by demineralized allograft covered by free autogenous periosteum. *Injury* 33:679–683
16. Christie J, Robinson CM, Pell AC et al (1995) Transcardiac echocardiography during invasive intramedullary procedures. *J Bone Joint Surg Br* 77:450–455
17. Wenda K, Runkel M, Degreif J et al (1993) Pathogenesis and clinical relevance of bone marrow embolism in medullary nailing—demonstrated by intraoperative echocardiography. *Injury* 24 (Suppl 3):S73–S81
18. Pape HC, Auf'm Kolk M, Paffrath T et al (1993) Primary intramedullary femur fixation in multiple trauma patients with associated lung contusion—a cause of posttraumatic ARDS? *J Trauma* 34:540–547, discussion 547–548
19. Kim DH, Rhim R, Li L, Martha J, Swaim B, Banco RJ, Jenis LG, Tromanhauser SG (2009) Prospective study of iliac crest bone graft harvest site pain and morbidity. *Spine J* 9:886–892
20. Schwartz CE, Martha JF, Kowalski P, Wang DA, Bode R, Li L, Kim DH (2009) Prospective evaluation of chronic pain associated with posterior autologous iliac crest bone graft harvest and its effect on postoperative outcome. *Health Qual Life Outcomes* 7:49
21. Cockin J (1971) Autologous bone grafting—complications at the donor site. In: *Proceedings of the British Orthopaedic Association*. *J Bone Joint Surg Br* 53:153
22. Bovill DF, Skinner HB (1989) Unicameral bone cysts. A comparison of treatment options. *Orthop Rev* 18(4):420–427
23. Gautschi OP, Cadosch D, Frey SP, Skirving AP, Filgueira L, Zellweger R (2009) Serum-mediated osteogenic effect in traumatic brain-injured patients. *ANZ J Surg* 79(6):449–455