Long Term Follow-Up of Pediatric Mandibular Reconstruction With Human Transforming Growth Factor-β3

Ferretti, Carlo BDS, MDent (MFOS) 1,2 and Ripamonti, Ugo MD, PhD2,*

1 Bone Research Laboratory, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences; and 2Department of Maxillofacial and Oral Surgery, University of Pretoria, formerly School of Oral Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

*Address correspondence and reprint requests to Ugo Ripamonti, MD, PhD, Bone Research Laboratory, Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, 7 York Road, Parktown 2193, South Africa; E-mail: ugo.ripamonti@wits.ac.za

Abstract

Translating bone regeneration induced by recombinant human bone morphogenetic proteins from animal models to human patients has proven inexplicably inconsistent. This prompted us to test in 5 pediatric patients, an alternative osteoinductive morphogen, recombinant human transforming growth factor β3 (hTGF- β3), to reconstruct mandibular defects of such a size to preclude reconstruction with autologous bone. An osteoinductive implant of human demineralized bone matrix (DBM) loaded with 125 μg hTGF- β3 per gram of DBM was implanted into one defect, and 250 μg hTGF-β3 per gram of DBM in another. Thereafter in 3 patients limited amounts of particulate cortico-cancellous bone graft harvested from the posterior iliac crest were combined with 250 μg hTGF- β3 per gram of DBM. Patients were followed up for 3 to 6 years. Three patients achieved clinically significant osteoinduction, 1 patient with hTGF- β3 only, and 2 by combining hTGF- β3 with a small supplement of autologous bone. One patient with hTGF- β3 only and followed up for 5 years retains a viable reconstruction but has had sub-optimal bone regeneration. One patient had osteoinductive failure due to sepsis although the plate reconstruction remains viable. Recombinant human TGF- β3 initiates osteoinduction in humans and potentiates autologous bone graft activity allowing the reconstruction of large mandibular defects in pediatric patients.

Key Words: Bone morphogenetic proteins, human, mandible, tissue engineering, transforming growth factor- β3

The reconstruction of a large osseous defect in a pediatric patient is challenging as the usual sources of autologous bone seldom yield sufficient bone volume. Whilst it is a well-recognized phenomenon that sporadically and unpredictably a mandible may regenerate spontaneously in a child, this phenomenon cannot be relied upon to obviate the need for formal reconstruction.1 Although the dimensions of the pediatric fibula make it unsuitable to reconstruct a mandible and the resultant reconstruction does not replicate mandibular morphology, it remains the work horse of pediatric mandibular reconstruction.2 This is due to the mistaken belief that osseous defects longer than 6cm cannot be reconstructed with a non-vascularized graft and surgical enthusiasm for free flap reconstruction. This imposes on a young patient increased morbidity, costs, and (especially in children) a reconstruction which fails to replicate mandibular anatomy. Reconstruction methods which reduce or replace the need for autologous bone, would greatly improve the quality of life of patients. Therapeutic bone tissue engineering in human patients exploiting the ‘bone induction principle’ was first described by Marshall Urist.3,4 The extraction and purification of naturally-derived bone morphogenetic proteins (BMPs)5,6 led to the cloning and production of recombinant human BMP-2 (hBMP-2)7 and hBMP-7.8 Naturally-derived and recombinant
hBMPs, healed critical size experimental surgical defects in many animal species (for review see 9,10), and persuaded clinicians to attempt clinical bone tissue engineering in humans. The first human trials of bone tissue engineering using naturally derived BMPs focused on long bone non-union.11 Recombinant human BMPs (rhBMPs) have been used predominantly in long bone non-union and spinal fusion.13 In the latter scenario it became apparent that the use of rhBMPs was accompanied by many complications over and above the failure of fusion.14,15 The use of naturally derived BMPs and off-label use of rhBMPs [hBMP-2 (Infuse, Medtronic) and hBMP-7 (Osigraft, Stryker Biotech)] for the reconstruction of major defects of the maxillofacial skeleton was described in several case reports and case series producing inconsistent results.16–22 A hypocellular and hypovascular recipient site incapable of responding to an implanted growth factor may be one of the factors leading to osteo-inductive failure. Three cases have been reported that endeavored to circumvent a compromised recipient site by first implanting an osteoinductive implant in a healthy heterotopic site (latissimus dorsi, pectoralis major and omentum) and then transplanting to the mandibular recipient site.23–25 Even when implanted in these more favorable sites, only histologically detectable bone formed, ossicle formation failed and reconstruction failure ensued.23–25 To these authors’ credit these complications were reported, but one nevertheless cannot overlook that the bone regeneration achieved (even in a healthy heterotopic site) had failed therapeutically.23,26,27 Moreover, this technique undermines the dominant rationale for the use of bone tissue engineering; reduction in surgical morbidity, treatment time, costs, and avoidance of a donor site.

Despite the failure of hBMPs to meet the minimum requirement of equivalence with existing therapy, most studies concluded with an enthusiastic endorsement of bone tissue engineering, partly the consequence of two factors: using maintenance of osseous segment stability (a function of the implanted metal hardware) as a proxy for osteoinductive success, and the reliance on histology (often at high power) to identify bone tissue. The identification of bone tissue on high power histology masks the failure of bone organ restitution. Routine radiographic examination must be used to determine whether the regenerated bone can be identified radiographically as normal bone (in radiodensity and trabecular architecture), and the reconstruction morphologically resembles the former skeletal segment.28 Important secondary characteristics are the loss of the bone/implant interface and the absence of residual delivery matrix. In the absence of these hallmarks, bone tissue engineering cannot be deemed to be successful. Thus, the ongoing use of only one of the osteoinductive members of the transforming growth factor-β family to treat any defect of the human skeleton is no longer driven by evidence but expediency.

Transforming growth factor-β (TGF-β), a pleiotropic polypeptide abundant in bone matrix, is a key participant in osteoblast and bone biology and regulates the composition and mechanical properties of bone matrix.29,30 TGF-β initiates chondrogenesis and osteogenesis in vivo after sub-periosteal injection on the parietal bones and femurs of neonatal rats.31,32 In the early stages of osteoblastic cell differentiation, mammalian TGF-β isoforms act upstream of BMPs as a master regulator of mesenchymal differentiation.33 Thus, whilst BMPs are the final common pathway responsible for osteoblastic differentiation and the induction of bone formation, TGF-β is the controlling initiator. In the last 2 decades our laboratories have investigated osteoinduction in non-human primates by the three mammalian TGF-β isoforms, and particularly recombinant human TGF-β3 (hTGF- β3).34 In the Chacma baboon (Papio ursinus), hTGF- β3 implantation in intramuscular sites induces vigorous and substantial bone formation.33,34 The performance advantage of hTGF- β3 over hOP-1 in heterotopic sites was also confirmed in systematic studies in Papio
Additional studies showed unequivocally that the induced mineralized bone and osteoid volumes, alkaline phosphatase activity and calcium content of the harvested ossicles after heterotopic intramuscular implantation of 5 μg hTGF-β1 in *Papio ursinus* was greater than 25 μg hBMP-7.10 Finally 125 μg of TGF-β3 per centimeter of experimental segmental mandibular defect in *Papio ursinus* induced vigorous ossification and bridging of the defect by day 30.35 The use of hTGF-β3 was never associated with local or systemic adverse events. This report describes our experiences with the use of hTGF-β3 to reconstruct large mandibular defects in 5 pediatric patients.

**MATERIALS AND METHODS**

Five patients (between the ages of 8 and 13 years) were treated for mandibular tumors by mandibular resection. Standard reconstructive options were not feasible due to the insufficient volume of autologous bone available for such large reconstructions. Following discussions with members of the Human Research Ethics Committee of the University, permission was granted for humanitarian use (HREC (Medical) Clearance certificate no. M170597). The patient's parents were informed, exhaustively counseled and all confirmed willingness to proceed by signing an informed consent form.

**Recombinant hTGF-β3 and Preparation of Osteogenic Devices**

Mature recombinant hTGF-β3 (Novartis AG, Basel, Switzerland), is a glycosylated 25-kDa homodimer with a C-terminal domain of 112 amino acids with nine cysteine residues.34 Human demineralized bone matrix (DBM) was supplied as 1 g aliquots in glass vials (Pretoria Tissue Bank, Pretoria, South Africa).

To each vial was added (for the first patient) 125 μg of hTGF-β3 and the remaining four patients 250 μg of hTGF-β3 in solution. The reconstituted matrix was then lyophilized and gamma-irradiated awaiting implantation.

**Surgery**

The surgical technique was the same for all patients. In the patients whose tumor had perforated mandibular cortical bone, expanded into soft tissue, and if tumor biology dictated, the resection included periosteum, overlying muscle and portions of oral mucosa. We have previously described the intermediate reconstruction phase of treatment.36 To summarize, a pre-bent, patient matched mandibular plate (Biomet Microfixation, Jacksonville, FL) was secured to the extant mandible and a medical grade silicone spacer was secured to the reconstruction plate (Fig. 1A). Eight weeks later definitive reconstruction ensued. The harvested posterior iliac crest bone was milled in a power bone mill to create a particulate cortico-cancellous bone (PCCB) graft. The defect was exposed via an extraoral incision placed in the first neck crease. The silicone spacer was exposed and explanted, and the osseous interfaces of the extant mandible segments were debrided of adhering soft tissues. In those patients in whom the mandibular condyle had been resected a costo-chondral graft was harvested (from the right fourth or fifth rib) and secured to the plate, to reconstruct the condyle ramus subunit (Fig. 1B). The remaining defect was reconstructed either with the osteoinductive implant mixed with saline solution to form a paste (in 2 patients) (Fig. 1C) or, mixed with particulate cortico-cancellous bone (PCCB) graft (in three patients – Fig. 1D). This was loaded into a syringe and extruded into the defect.
Description of Patients

1. Eight-year-old male with odontogenic myxoma of the right ramus and body of the mandible (E). The right hemi-mandible was resected in a sub-periosteal plane with portion of overlying oral mucosa (F). Three months later the 11 cm long defect of the mandible was reconstructed with a 5 cm costo-chondral graft for the condylar head and vertical ramus, and 6 g of human DBM loaded with 750 μg hTGF-β3 for the body of the mandible (C).

2. Eight-year-old male with a large aneurysmal bone cyst of the left mandibular ramus and body (A). The left hemi-mandible was resected in a sub-periosteal plane (B). Three months later the 11 cm long defect of the mandible was reconstructed with a 6 cm costo-chondral graft for the condylar head and vertical ramus, and 12 g of human DBM loaded with 2500 μg hTGF-β3 for the body of the mandible.

3. Thirteen-year-old male with ameloblastoma extending from the right first premolar to the left second molar (C) requiring supraperiosteal resection of 13 cm portion of mandible (D). Three months later the mandible was reconstructed with 14 g DBM loaded with 3500 μg hTGF-β3 mixed with 26 cc (52 g) of compressed PCCB graft.

4. Eleven-year-old female with a mandibular ameloblastoma extending from right second molar the left second mandibular molar requiring supra-periosteal resection of a 14 cm portion of mandible (E). Three months later the mandible was reconstructed with 12 g of PCCB graft from the posterior iliac crest mixed with 15 g DBM loaded with 3750 μg of hTGF-β3.

5. Thirteen-year-old male with mandibular ameloblastoma from the right condyle to the right first premolar (F) necessitating supra-periosteal resection of an 11.5 cm mandibular segment. Three months later the mandible was reconstructed with a 6.5 cm portion of costo-chondral graft for the condylar head and vertical ramus and 13 g of PCCB graft from the posterior iliac crest mixed with 10 g DBM loaded with 2500 μg hTGF-β3 for the mandibular body.

Follow-up, Radiographic Assessment and Determinants of Success

All patients were followed at regular intervals and examined clinically and radiographically. Success would be determined above all by the radiographic appearance of the implant. The radiographic hallmarks of clinically significant osteoinduction would be coalescence of radio-opacities into a single ossicle with radio-density similar to adjacent bone, trabecular patterns clearly visible in the regenerated bone, and defect-host bone interface no longer visible.

RESULTS

None of the patients suffered untoward systemic effects following the implantation of the osteoinductive device.

Patient 1 - Followed up for 5 years. The reconstruction has survived, and facial symmetry has been maintained. Radiographic examination shows scattered radio-opacities consistent with bone regeneration within the defect consistent successful osteoinduction. However, restitution to integrity of the osseous defect was not achieved (Fig. 3A).
FIGURE 3. A, Panoramic radiograph of mandible of patient 1, 5 years post reconstruction (costo-chondral graft and hTGF-β3 solo). Scattered islands of bone within the defect. B, Panoramic radiograph of mandible of patient 2, 6 years post reconstruction (hTGF-β3 solo). Complete osseous regeneration within the mandibular defect and restoration of mandibular morphology. C, Panoramic radiograph of mandible of patient 4, immediately post reconstruction with hTGF-β3 - DBM mixed with 13 grams of autologous bone graft. The implant is faintly radiopaque and clearly granular. D, Panoramic radiograph of mandible of patient 4, 6 years post reconstruction. The implant has coalesced into a trabeculated, mature bone ossicle spanning the erstwhile defect site. The original resection margins are no longer visible. E, Panoramic radiograph of mandible in patient 5, 3 years post reconstruction. The defect has been bridged by an ossicle of mature bone.

Patient 2 – Followed up for 6 years. The reconstruction has survived, facial symmetry has been maintained. Mandibular form has been restored, and bone regeneration within the defect is clearly evident in the radiographic examination (Fig. 3C).

Patient 3 - Sepsis developed 2 months following implantation and the entire implant was removed. The reconstruction plate was left in situ and the patient continues to function with the reconstruction plate.

Patient 4 – Followed up for 6 years. The reconstruction has survived and facial symmetry has been maintained. The defect is bridged by clearly identifiable mature bone. Osseointegrated
implants have been placed and the patient rehabilitated with a fixed dental prosthesis (Fig. 3C and D).

Patient 5 – Followed up for 3 years. The reconstruction has survived but facial growth has been asymmetrical. The defect has been bridged by an ossicle of mineralized bone (Fig. 3E).

DISCUSSION

The prospect that tissue engineering may facilitate the reconstruction of massive skeletal defects in pediatric patients has yet to be explored. Although it is patent that the need for bone tissue engineering is more pressing in massive skeletal defects of children than adults, the unknown effects of implanting large doses of recombinant osteoinductive morphogens in a growing patient deterred early adoption of the available biotechnology. Unfortunately, even in adult patients the published results are discouraging. Only a few of the case reports and case series describing human bone tissue engineering using hBMPs can claim clinical results equivalent to autologous bone grafts.

This study investigated osteoinduction mediated by a novel hTGF-β3 osteogenic device (which has shown great promise in non-human primate models), in a cohort of pediatric human patients. The 5 pediatric patients treated all had major defects of the mandible with no prospect of successful reconstruction with autologous tissue transfers.

Two fundamental issues must be considered when assembling an osteoinductive implant: growth factor dose, and delivery system or carrier. Reciprocal stimuli between the morphogen, delivery matrix and recipient bed, profoundly affects the osteoinductive response. The dose of osteoinductive morphogen must to some degree be based on data gleaned from animal trials, with the caveat that osteoinductive growth factor dose is species specific, and even large primate animal models do not accurately replicate the bone physiology of Homo sapiens. Indeed clinicians have been repeatedly confounded by the disparity that exists between osteoinduction by hBMPs in animal models and in human patients. Nevertheless, dose response studies of hBMPs have determined that osteoinduction does not occur below a threshold dose, tissue response increases as growth factor dose increases, and no further benefit accrues above an upper threshold dose. A dose for clinical application should be just below the upper threshold and low enough to avoid undesirable side effects. Recombinant hTGF-β3 loaded on baboon insoluble collagenous bone matrix (ICBM) was first tested in the Chacma baboon Papio ursinus at doses of 5, 25, and 125 μg hTGF-β3. Bone induction in heterotopic sites was most vigorous with the 125 μg. In additional heterotopic experiments, 250 μg hTGF-β3 delivered by sintered ceramic macroporous constructs showed prominent induction of bone formation extending beyond the perimeter of the implanted bioreactors.

Bone tissue engineering requires the spatial and temporal control of osteoinductive growth factor diffusion and is affected by the physical properties of the carrier. A carrier should be easily adapted to a defect, provide immediate structural support to the reconstructed bone, and elicit a suitable and commensurate immunological response. Once implanted, it should exert temporal control over diffusion of the selected morphogen. Finally, resorption of the delivery system should be coupled to the advancing osteogenetic front.

The most commonly used delivery for hBMP-2 has been Collagen Type 1 sponge. Its main drawback is that it lacks compression resistance and is one of the reasons that the
resultant ossicles are volumetrically deficient. Xenogeneic hydroxyapatite has been used as a bone substitute and to deliver osteoinductive morphogens.23–25 Although it enjoys superior compression resistance it is extremely impervious to resorption and thus impedes bone regeneration. We have used human DBM in our previous work to deliver naturally derived BMPs.18 Human DBM resorbs readily and is weakly osteoinductive, (but thoroughly inadequate to treat large skeletal defect solo) and contains several osteoinductive growth factors which may synergize with the added hTGF- β3.

Based on our earlier experimental work on the Chacma baboon,35 the dose selected for the first patient was 125 μg hTGF- β3 per gram of human DBM. One gram of device was implanted per centimeter of mandibular defect length for a total dose of 750 μg hTGF- β3. This yielded scattered ossicles within the defect but failed to achieve clinically significant osteoinduction. Although this patient's reconstruction remains functional 5 years post implantation, from a tissue regeneration point of view the regenerated bone did not match the regeneration that one might expect with an adequate volume of autologous bone. We thus increased the dose for the subsequent patients to 250 μg hTGF- β3 per gram of human DBM. The second patient (total implanted dose 2500 μg hTGF- β3) obtained excellent regeneration of both the body and ramus, and the form of the regenerated ossicle recapitulated the mandibular anatomy. The volume of the regenerated bone far exceeded the volume of the implant and the costo-chondral graft.

Thus, our first 2 patients treated with hTGF- β3 loaded onto DBM only, yielded inconsistent results. In addition, the regular reports of suboptimal outcomes from other investigators using hBMP in humans, combined with our earlier work with naturally derived BMPs reinforced our misgivings that clinically significant osteoinduction could be achieved using only an inductive morphogen and an appropriate delivery system.16–27 If we consider the complex cellular, growth factor and mineral constituents of an autologous bone graft perhaps the expectations and optimism for single morphogen therapy are misplaced. We had to accept (as other surgeons have)39 that combining an osteoinductive implant with an ABG may improve clinical outcomes, but require a far lower volume of ABG. We acknowledge that combining an osteoinductive device with an ABG adds ambiguity to the assessment of the osteoinductive activity of a recombinant human morphogen. Prioritizing therapeutic success over experimental clarity obliged us to test hTGF- β3 as an autologous bone graft expander or potentiator, to improve the yields of bone regeneration. However, we have shown that to obtain a useful ossicle in mandibular reconstruction with a PCCB graft requires 6.9 grams of bone per centimeter of mandibular defect.40 The mass of bone graft used in our patients was roughly 20% of the mass normally required to reconstruct such defects. Using only the harvested PCCB would have resulted in failure of ossicle formation.

Two of the 3 patients who received the ABG mixed with the osteoinductive implant healed uneventfully. Both patients (followed up for 3 and 6 years) regenerated an ossicle across the defect that was unambiguously mature bone. Clinically significant bone regeneration was due to the addition of the hTGF- β3 osteoinductive implant. Graft potentiation with hTGF- β3 allows the reconstruction of defects which would otherwise not have been possible with the ABG volumes available in a child.

This clinical study reports data (after an above average period of follow-up for such studies) on the first human use of the osteoinductive morphogen hTGF- β3 in large mandibular defects in a selected group of pediatric patients. The osteoinductive growth factor hTGF- β3 in combination with DBM and implanted in human patients yielded confounding results;
excellent regeneration in 1 patient and sub-optimal in another. Our understanding of these variations is incomplete and at this juncture in the evolution of clinical bone tissue engineering it may be prudent to accept that it is unrealistic to expect a single osteoinductive morphogen deployed on a suitable carrier to predictably regenerate bone volumes comparable to an autologous bone graft. Combining the volume of bone typically available from a pediatric ilium with a hTGF-β3 allows the successful reconstruction of large mandibular defects in pediatric patients and is an alternative bone tissue engineering strategy that merits more in-depth clinical research.

Finally, our reported mechanistic insights on the induction of bone formation by the hTGF-β3 isoform in the non-human primate *Papio ursinus* offers a temporo-spatial molecular background behind the limited and substandard induction of bone formation by hBMPs in clinical contexts. Physiological expression of endogenous BMPs genes and gene products by the direct implantation of hTGF-β3 with TGF-β3 and BMPs gene expression may physiologically escape the antagonistic expression of Noggin, whereas the direct implantation of far too high doses of exogenously applied hBMPs in clinical contexts set into motion the immediate expression of Noggin and other inhibitory byproducts tightly controlling the bone induction cascade in humans, as shown by the limited effectiveness of hBMPs in clinical contexts. The results obtained in the non-human and human primates now warrant a re-evaluation of the induction of bone formation in primates by the pleiotropic osteogenic members of the TGF-β superfamily of proteins.

**Acknowledgment**

The authors thank Novartis AG for the recombinant protein.

**REFERENCES**


