



An ongoing research for evaluation of treatment with BMPs or AGFs in long bone non-union: Protocol description and preliminary results

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KEYWORDS

Non-union;
BMP-7;
Platelet rich plasma;
Tissue engineering

Summary Treatment of long bone non-union (NU) continues to be a challenging task for the trauma surgeon often resulting in unsatisfactory results and long-term morbidity. Whilst autologous bone grafting remains the gold standard of treatment of these difficult cases, recently, due to the advances made in tissue engineering techniques, other alternatives have become available. In this study we report our preliminary results of treating long bone non-unions using either bone morphogenetic protein-7 (BMP-7) or platelet rich plasma (PRP) concentrations. Twenty-nine cases have entered this study thus far. Preliminary results indicate that BMP-7 is more efficacious than PRP as there was a significant failure rate of 6.2% versus 38.5% between BMP-7 and PRP, respectively.

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Introduction

Post-traumatic non-union (NU) is one of the most complex pathologies to treat and sometimes require several corrective interventions: cases not resolved after the third surgery become extremely resistant to further treatment.

Several treatment methods have been proposed and it seems that the use of more than one technique at the same time may provide the best results. It has been widely proven that that some growth factors (GFs) act as stimulators for the proliferation of osteoblasts in vitro and for

the healing of bone in vivo and they have now considered useful in healing processes if correctly applied to the injury site.¹⁷

The continuous evolution in tissue engineering using recombinant DNA techniques have led to the production of BMPs, which are recognised today as the only osteoinductive factors¹¹. In parallel parameters have also been defined for the preparation of platelet rich plasma (PRP) a source of autologous growth factors (AGFs) contained in the platelets (PDGF, FGF, EGF, ILGF).^{12,13}

Considering both the growing experimental evidence claiming for a therapeutic potential of BMPs and AGFs and the relative paucity of controlled clinical studies we decided to initiate a wide

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research at our Institution in order to test the clinical and radiological efficacy of BMPs/AGFs in post-traumatic (long bone non-union and osseous defects) and reconstructive hip surgery (acetabular defects and prostheses revisions) plus in osteochondral defects.

The overall recruitment period will be 3 years during which 100 patients/year will be enrolled divided in the following manner:

Hip surgery: 40 cases/year \times 3yrs = 120 cases, 60 treated with rh BMPs and 60 with PRP

Long bone NU/critical size trauma defects: 40 cases/year \times 3yrs = 120 cases, 60 treated with rh BMPs and 60 with PRP

Osteochondral defects: 20 cases treated with PRP/year \times 3 years = 60

In the herein study the protocol and the Case Report Form (CRF) used for the patients suffering from long bone NU/osseous defects will be described and some preliminary results will also be reported.

Long bone NU/critical size bone protocol description

We are experimenting the use of AGFs and BMPs in patients suffering from non-reactive post-traumatic long bone NU/critical size bone defects in order to evaluate their efficacy, regardless of the used osteosynthesis in order to establish a real alternative to the use of Iliac Crest Bone Graft (ICBG). The research is a phase 3, investigator's driven clinical study.

The protocol was approved by the Ethical Committee at the Istituto Ortopedico Gaetano Pini, Milan University, the project is totally funded by the Lombardy region.

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Inclusion criteria

Two separate pathologies implies the randomized use of rhBMP-7 or PRP:

Group A

Patients affected by long bones non-union, 9 months minimum duration, who are judged not to heal by simply changing the osteosynthesis device.

Group B

Patients with non-neoplastic, post-trauma or post-resection osseous defects of a critical size that will

probably not heal using traditional surgical techniques or for which such techniques are considered to be unsuitable.

Before being enrolled in this study, the patient must supply his written, informed consent.

Exclusion criteria

The patients presenting one or more of the following criteria are excluded from the study:

Patients with high hypersensitivity to the active constituent or to collagen.

Pregnant women or women who may become pregnant during the study.

Patients whose skeleton is not yet completely formed.

Patients with an ongoing infection in the non-consolidated site or systemic infections in progress.

Patients with insufficient skin to cover the fracture site and insufficient vascularisation at site of non-consolidation.

Patients with non-consolidation due to pathological fractures (tumours, metabolic osteopathy). Patients with a tumour close to non-consolidation site.

Patients who have undergone chemotherapy, radiotherapy or immunosuppression

Patients with ascertained auto-immune disorders.

Patients affected by congenital pseudo-arthritis.

Patients who are not mentally capable or who would not follow the post-operation evaluation program.

Patients who have already undergone treatment with any rhBMP or PRP).

Patients undergoing chronic therapy with steroids or NSAIDS (non-steroid anti-inflammatory drugs).

Patients for whom an Autologous Bone Graft is considered absolutely necessary for healing.

Treatment

When this protocol was written and presently, the only rhBMP available in Italy is the rhBMP-7 (3.5 mg Eptotermin alfa, INN name) mixed to a bio-reabsorbable support (1 g of collagen) registered as a drug at European level (OsigraftTM Stryker).⁶

The drug is contained in a sterile vial and has to be reconstituted with 2–3 ml of saline just few minutes before use, as described in the Summary Product Characteristics (SPC); it is then implanted into the lesion during an open surgical operation, after applied the fixation device (IM nail, plate or

External Fixator). A maximum of two vials can be used.

The PRP is obtained at the same time of the surgery. Depending on the extent of the area to be treated, it is possible to obtain 10/20 ml of PRP (collecting 54/108 ml of peripheral blood), adding 12 ml of an anticoagulant. The blood is centrifuged twice for 14 min in order to divide the platelet poor plasma (PPP) from the platelet rich plasma. Once removed the PPP, the PRP is aspirated in a syringe.

A procoagulant taken from the patient's blood (2 ml) and centrifuged at the same time, is needed to activate the platelets; 0.3 ml of 10% calcium chloride is added to the procoagulant, this mix is then added with the PRP using an applicator. The coagulation process is completed in 4.6 min from the moment when it is applied.

Ten or 20 ml of platelet gel is added to the chosen graft. The platelet concentration in our PRP is 1.582 /nL.

Evaluation criteria—case report form description

Patient demographics, past medical history, and trauma history (1) date of the original trauma (2) trauma description (3) the presence of previous local pathologies (4) AO and Gustilo fracture classification (5) other injuries (fractures/soft tissue) linked to the trauma (6) classification of seriousness (ISS) (7) any previous treatments and bone grafts (specifying date and type of operation).

Current state of the patient (1) diagnosis (2) presence or absence of deformity (specifying type and number of degrees or centimetres of shortening) (3) pseudoarthrosis classification (Weber and Cech) i.e.: hypertrophic, oligotrophic, atrophic or loss of substance, the presence of osseous defect (specifying type and degree) (4) presence of infection using the Cierny Mader (ongoing, previous, resolved) classification.

Treatment (1) date (2) type of operation (3) operation duration (4) use rhBMP-7 or PRP and any associated bone grafting procedures (homologous, heterologous bone) (5) osteosynthesis device.

Adverse effects occurring during the operation, immediately after the operation or at a later date will also be registered.

Clinical and X-ray findings are registered pre-operative, intra-operative, before hospital discharge, post-operative (1, 3, 6, 9 and 12 months) and thereafter (optional, according to the specific case). An overall evaluation of progress where indicated will be recorded for each check-up.

Clinical evaluation

1. *Pain*: at rest, under weight bearing
2. *Functionality*: none/insufficient; use against gravity; use against resistance; full
3. *Walking*: not possible; possible with orthopedic devices; limp; partial (indicate % bearing allowed); full

Table 1 Demographics of patients randomised to rhOP-1 and PRP

	rh OP-1	PRP
Number of Patients (9 mos Follow up)	16	13
Age (y) mean \pm SD	47.4 \pm 2.56	35.3 \pm 1.76
Atrophic NU	7/16	6/13
Site NU	6 Tibia 5 Femur 2 Humerus 2 Ulna 1 Radius	4 Tibia 2 Femur 3 Humerus 2 Ulna 2 Radius
NU duration (months) – mean \pm S.D.	15.2 \pm 2.46	18.8 \pm 3.02
No. previous surgery – mean \pm S.D.	2.5 \pm 0.57	2.6 \pm 0.66
Synthesis change %	4/16 (25%)	8/13 (61.5%)
RX healing rate	15/16 (94%)	8/13 (61.5%)
RX healing time (months) – mean \pm S.D.	8 \pm 0.43	9 \pm 0.49
Clinical healing rate	15/16 (94%)	8/13 (61.5%)
Clinical healing time (months) – mean \pm S.D.	3.5 \pm 0.41	4 \pm 0.56
Failures	1/16 (6.2%) ^a	5/13 (38.5%) ^b
Re-intervention (done/planned)	1/16 (6.2%)	3/13 (23%)

^a New trauma with Ex Fix failure and re-fracture.

^b First case: sepsis; second case: absence of callus formation; third case: new fracture in severe osteopenia; fourth and fifth cases: poor callus formation.

4. *Tests*: site instability; abnormal motility; site stability but painful; full site stability, pain-free
5. *Muscular trophism*: hypo/normo/hypertrophic (indicate cm circumference);

3. Callus staging: initial connections, bridging callus, advanced callus, mature callus (union).

Radiological evaluation

1. Presence of callus
2. Type of callus/bone repair: Inter-fragmentary, periosteal, endosteal, regenerated

Results

Patient recruitment began in April 2005, therefore a forecast has been made of a recruitment of 120 patients by April 2008.

To date, a total of 38 patient have been treated at the Istituto Gaetano Pini; 29/38 patients can be



Figure 1 (a) Female, 50 years, medium obesity: bifocal right femoral fracture (basicervical and diaphyseal), treatment with IMN static. Two years later: bifocal non-union at femur neck with angular deformity and femur diaphysis. (b) Intra-Op: neck calloclasia and cervico-cefalic deformity correction; renailing with static gamma-long + 2 vials osigraft only at femoral neck site. (c) 3 months post-Op: clinical evaluation: no pain; full weightbearing; full r.o.m. RX: sup-post-lat bone bridging at femoral neck, no variation at diaphysis. (d) 7 months: clinical evaluation: normal; RX femoral neck: callus remodelling; no callus progression at diaphysis. (e) 12 months post-OP: clinical evaluation: normal; RX: healing (bone remodelling) at femoral neck, NU persistence at femoral diaphysis.



Figure 1. (Continued).

evaluated as they have completed the minimum follow-up period of 9 months.

Sixteen of whom have been treated with rh OP1 and 13 treated with PRP.

The preliminary results obtained are descriptively reported in Table 1. Any statistical evaluation wasn't performed to date because of the limited number of patients.

Evident differences in healing /failure rate in the two groups are observed.

Fig. 1 report one BMP-7 case, female, 50 years, medium obesity with a bifocal right femoral fracture (basi-cervical e diaphyseal), treated with static IM-Nail; 2 years after diagnosis of bifocal non-union at neck femur with angular deformity and non-union at diaphysis. Treatment: neck calloclasia and

cervico-cefalic deformity correction; renailing with static gamma-long + 2 vials Osigraft at neck femur, none at diaphyseal level.

Healing at 12 months of neck femur, NU persistence at diaphyseal level.

This case show different evidence of healing in the same patient in the same bone according to BMP 7 use

Fig. 2 report one PRP case: exposed bifocal fracture right forearm at ulnar diaphysis with



Figure 2 Case PRP: (a) Female, 22 years: June 2004: exposed bifocal fracture right forearm; fracture right ulna dislocation right proximal radius; subcutis exposed; treatment with EF, deformity: 20° valgus. (b) Intra-op: At 9 months: Ulnar non-union: treatment with intramedullary nail + platelet rich plasma. (c) 1 month post-OP: CL: no pain—good r.o.m. RX: bridging callus. (d) 12 months post-OP: CL: normal— RX: remodeling.



Figure 2. (Continued).

dislocation proximal radius; subcutis exposed; treatment with EF, deformity: 20° valgus. At 9 months non-union ulna: treatment with intramedullary nail + platelet rich plasma; at 1 month post-Op RX: bridging callus, at 12 Months post-Op RX:healing (bone remodelling).

Discussion

Bone grafting is one of the most frequent types of transplants, second only to blood transfusions; an approximate estimate of procedures carried out is about 600,000 per year in Europe, with iliac crest bone graft (ICBG) used in 60% of all procedures. Autologous bone graft is currently considered the "golden standard" for pseudoarthroses and bone defects because of osteoconductive (mineral

content and collagen) osteoinductive (BMPs) and osteogenetic (mesenchymal cells) properties.

Due to complications at the donor site,^{9,10,22} the relative associated health costs¹⁸ and the patient's discomfort (chronic pain),¹⁰ research is constantly looking for valid alternatives.

The combined use of osteoconductive scaffolds, osteoinductive (BMPs) or osteoproliferative (AGFs)-stimuli and/or osteoprogenitor cells is emerging in order to successfully replace the need to harvest autologous bone.

The discovery of growth factors, BMPs in particular, determined notable progresses in knowledge of the physiology and reparative mechanisms of bone. These growth but mainly differentiation factors demonstrated the unique property to stimulate the formation of new bone, even in extra-skeleton sites.¹⁹

In the field of traumatology, non-union in particular, BMPs have the largest amount of evidence of efficacy in controlled randomized study⁷ and case series^{1,2,4,5,8,14,16,20} also implying recalcitrant, difficult to treat NU.

For the AGFs contained in PRP, preclinical data show that they are unable to differentiate the mesenchymal cell into osteoblasts (osteoinductivity), to increase new bone formation when associated with osteoconductive agents and to cause heterotopic ossification¹⁵ even if they have been suggested to repair large bone defects.

In spite of the rather popular use, there is to date a lack of significant clinical documentation in orthopaedic/traumatology field (pseudoarthrosis in particular); not encouraging results were recently reported in spine surgery^{3,21} even if its use in CMF (Cranio-Maxillofacial) applications is well documented. The concentration of platelets/AGFs, which may vary depending on the various techniques used for PRP preparation, seems to be the crucial element to influence the clinical outcome.¹³

The recombinant BMPs available, registered in Europe as pharmaceuticals, are rh BMP-7 (or rhOP-1, Eptotermin alfa INN), carried by type 1 Collagen (the only now available in Italy, therefore the only used in our experiment) and rhBMP-2 (INN Diboterminal alfa INN) absorbable on a collagen sponge.

rhBMP-7 is currently the first and only BMP approved in non-union of long bones refractory to autograft (tibial non-union in Europe)⁶ and for spinal non-union (in USA as HDE, Humanitarian Device Exemption) with an efficacy similar to ICBG with better tolerability.

In light of these considerations, we started the above described study that, when completed, may provide useful information on the real therapeutic potential of both AGFs and BMPs as possible alternatives to ICBG in various fields of application.

According to our initial results, a relevant difference in healing/failure rate between rhBMP-7 and PRP is observed, even if a definitive conclusion isn't possible at this stage.

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