Clinical and Experimental Rheumatology 2008; 26: 910-913.

Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study

M. Sánchez[1], E. Anitua[2], J. Azofra[1], J.J. Aguirre[2], I. Andia[2]

[1]Unidad de Cirugia Artroscópica "Mikel Sanchez", Vitoria, Spain; [2]Biotechnology Institute, BTI IMASD, Vitoria, Spain.

Mikel Sánchez, MD
Eduardo Anitua, MD
Juan Azofra, MD
José Javier Aguirre, MD
Isabel Andia, PhD

The work of this group is partially funded by the Basque and Spanish Governments.

Please address correspondence and reprint requests to:
Dr. Isabel Andia, Biotechnology Institute, c/ Leonardo Da Vinci 14, 01510 Miñano (Alava), Spain.
E-mail. isabel.andia@bti-imasd.com

Received on June 18, 2007; accepted in revised form on February 6, 2008.

Key words: Osteoarthritis, platelets, growth factors, hyaluronan, WOMAC.

Conflict of interest:
Drs. E. Anitua, J.J. Aguirre and J. Azofra work in the research department of Biotechnology Institute, a dental implant company that commercialises a system for preparing platelet-rich plasma for therapeutic use; the other co-authors have declared no competing interests.

ABSTRACT

Objective. *To obtain preliminary information about the effectiveness of intra-articular injections of an autologous preparation rich in growth factors (PRGF) for knee OA treatment to be explored further in future studies.*

Methods. *We have characterized PRGF treatment by platelet count and concentration of relevant growth factors (TGF-β1, PDGF-AB, VEGF-A; HGF and IGF-I) involved in healing mechanisms. We have performed an observational retrospective cohort study using hyaluronan injections as a control. Each group included 30 patients with OA of the knee, matched according to age, sex, body mass index and radiographic severity. Both treatments were based on three weekly injections. Clinical outcome was examined using the WOMAC questionnaires prior to treatment and at 5 weeks after treatment.*

Results. *The observed success rates by week 5 for the pain subscale reached 33.4% for the PRGF group and 10% for the hyaluronan group. The difference was attributed exclusively to the treatment modality, p=0.004. The percent reductions in the physical function subscale and overall WOMAC at 5 weeks were also associated solely with treatment modality in favour of PRGF, p=0.043 and p=0.010 respectively.*

Conclusions. *Although these preliminary results need to be evaluated in a randomized clinical trial, they provide useful information about the safety of PRGF and open new perspectives on autologous treatments for joint diseases.*

Introduction

The treatment of osteoarthritis (OA) includes a wide spectrum of approaches but at present, with the exception of surgery, these approaches are merely palliative. Current research efforts towards the testing of protein biotherapeutics for restoring the metabolic balance within the capsular joint are in progress (1). Although the therapeutic use of anabolic and anti-catabolic factors appears promising, developing controllable approaches for delivery represent a major challenge (2).

Autologous platelet-rich fibrin (prepared from platelet-rich plasma) is a biological delivery system of a complex mixture of bioactive proteins essential to natural repair including anabolic factors for cartilage such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF) and insulin-like growth factor (IGF-I) (3). The potential of a characterized platelet-derivative, known as "Preparation Rich in Growth Factors" (PRGF), to enhance the limited capacity of cartilage to repair itself (4) encouraged the idea of treating degenerative joint conditions with this autologous preparation. Supported by the positive effects of PRGF in different clinical situations involving connective tissues (5-8) and also in OA synovial cell cultures (9), we have hypothesized that the delivery of a natural mixture of biologically active molecules within the joint compartment is a safe strategy (10) to induce positive changes in the joint microenvironment improving the conditions of articular tissues.

The goal of this preliminary study is to explore whether PRGF could be used for the treatment of OA of the knee. To address this issue, a full quantitative characterization of PRGF was carried out and a retrospective cohort study was performed to test the effectiveness and safety of PRGF. Since intra-articular hyaluronan (HA) treatments are also included among the therapeutic modalities for knee OA, we were able to study and compare two groups of patients: those who underwent conventional HA infiltration and those who underwent infiltration with PRGF.

Patients and methods

Patients

The study was designed as an observational retrospective cohort study using HA as a control. All patients signed a detailed informed consent. The study was conducted following the ICH GPC guidelines. Patients were diagnosed according to the American College of Rheumatology criteria (11). Radiographic severity was assessed by anterio-posterior weight bearing radiographs scored for Ahlbäck. Idiopathic and secondary post-traumatic and mechanical OA were included. OA secondary to joint inflammatory

Table I. Baseline characteristics of the studied patients.

	Hyaluronan	PRGF
Age	60.90 ± 8.63	63.53 ± 8.91
Percentage of Female	60	66
BMI (kg/m^2)	27.76 ± 4.87	29.95 ± 5.01
Ahlbäck grade (n)		
I	9	9
II	10	10
III	2	2
IV	9	9
WOMAC		
Pain	6.27 ± 6.57	8.40 ± 6.10
Stiffness	3.20 ± 3.07	3.63 ± 2.90
Physical function	22.87 ± 24.50	26.43 ± 22.33
Global	32.33 ± 34.13	38.47 ± 31.33

PRGF: preparation rich in growth factors; BMI: body mass index; WOMAC: Western Ontario McMasters University Osteoarthritis Index.

disease was excluded. Patients with other diseases affecting the knee, those with generalized OA or arthroscopic lavage in the year previous to treatment, or intra-articular treatment within the previous three months were excluded. The study included a total of 60 patients. Thirty patients received PRGF intra-articular injections while a similar group receiving HA was identified during the same time period. Patients in both groups were matched by age, gender and Body- Mass-Index (BMI) and also by radiographic severity (same Ahlbäck grade) (Table I). Concurrent medication such as paracetamol up to a maximum of 3 grams daily was permitted and discontinued 48h prior to WOMAC assessment.

PRGF

The preparation rich in growth factors is elaborated according to PRGF technology (class IIb medical device, Directive 93/42/EEC). Briefly, 34 cc of peripheral blood was collected into 9 cc tubes containing 3.8% (wt/vol) sodium citrate. Tubes were centrifuged at 640g for 8 min and the 2 cc plasma fraction located just above the buffy coat was aspirated and dispensed into an empty tube under vertical air flow conditions. Few minutes prior to the infiltration, calcium chloride was added at a final concentration of 22.8 mM. The activated liquid is injected before coagulation and so the fibrin scaffold containing the platelet aggregates is directly formed within the joint capsule. The delivery of proteins through this fibrin vehicle prevents leakage out of the joint.

The knee joint was entered by a lateral approach and 6-8 cc of activated PRGF were injected. The comparison group was injected with 2 cc of HA, (Arthrum H 2%, LCA Pharmaceutical, Chartres France). Both treatments were administered in series of three intra-articular injections at one-week intervals.

For the *in vitro* characterization, a platelet-rich fibrin matrix was formed by adding calcium chloride at a final concentration of 22.8 mM; clots were allowed to retract for one hour at 37ºC. In these test conditions, the fibrin scaffolds are fully retracted and the released supernatants contain the bulk of soluble proteins including PDGF-AB, TGF-β1, VEGF, IGF-I and HGF. All these factors were quantified in the supernatants using commercially available enzyme-linked immunosorbent assay kits (Quantikine colorimetric ELISA kits, R&D, Minneapolis, MN, USA).

Outcome evaluation

Patients filled out the validated Spanish version of the WOMAC questionnaire (12) (Western Ontario and McMaster Universities Osteoarthritis Index) at baseline and 5 weeks after the third injection. The primary efficacy criterion was changes from baseline in joint pain, measured using the WOMAC subscale (Likert format). Secondary efficacy variables included changes in joint stiffness, physical function and global WOMAC. Success rates were calculated according to a reduction in the WOMAC pain score of at least 40% from baseline. The results were evaluated by a masked observer.

Statistical analysis

Data are expressed as mean ±SD, unless otherwise indicated. Correlations were sought using the Pearson correlation coefficient. A factorial ANOVA model adjusted by BMI, age, sex and Ahlbäck grade was used to examine percent changes in subscales and global WOMAC. A p-value of 0.05 or less was considered significant. Statistical analyses were performed using SPSS version 15.0 (SPSS, Chicago, IL, USA).

Results

Characterization of PRGF therapy

The PRGF performed as described above resulted in a moderate enrichment in platelet number, a 2.0±0.5-fold increase compared to peripheral blood. White blood cell content in PRGF was below the detection limit of the haematological analyser (MICROS 60, ABX, Abingdon, UK), confirming the absence of leukocytes in the PRGF which improves the homogeneity of the product.

The levels of the main platelet secretory growth factors were 29.15±12.88 ng/cc (range, 8.39-57.55 ng/cc) for TGF-β1 and 17.41±9.66 ng/cc (range, 3.66-46.72 ng/cc) for PDGF. VEGF was also secreted from platelets but was less abundant (212 pg/cc, range 18-447 pg/cc). Other GFs present in PRGF reflect mainly plasma levels, among these growth factors are IGF-I (54.85±18.41 ng/cc, range 22.0-85.9 ng/cc) and less concentrated HGF (522±253 pg/cc, range 227-1115 pg/cc). Both IGF-I and TGF-β1 levels negatively correlated with age (p=0.005 and p=0.027, respectively).

Clinical outcome of PRGF and HA groups

A significant change from baseline in the WOMAC pain subscale was attributed to treatment modality, p=0.004. The observed success rates by week 5 for the pain subscale reached 33.3% for the PRGF group and 10% for the

control group. The changes from baseline in the physical function subscale and overall WOMAC were also associated with treatment modality, p=0.043 and p= 0.010 respectively (Fig. 1).
Mild injection pain and inflammation of short duration was reported by some patients and re-accumulation of effusion occurred commonly in both groups.

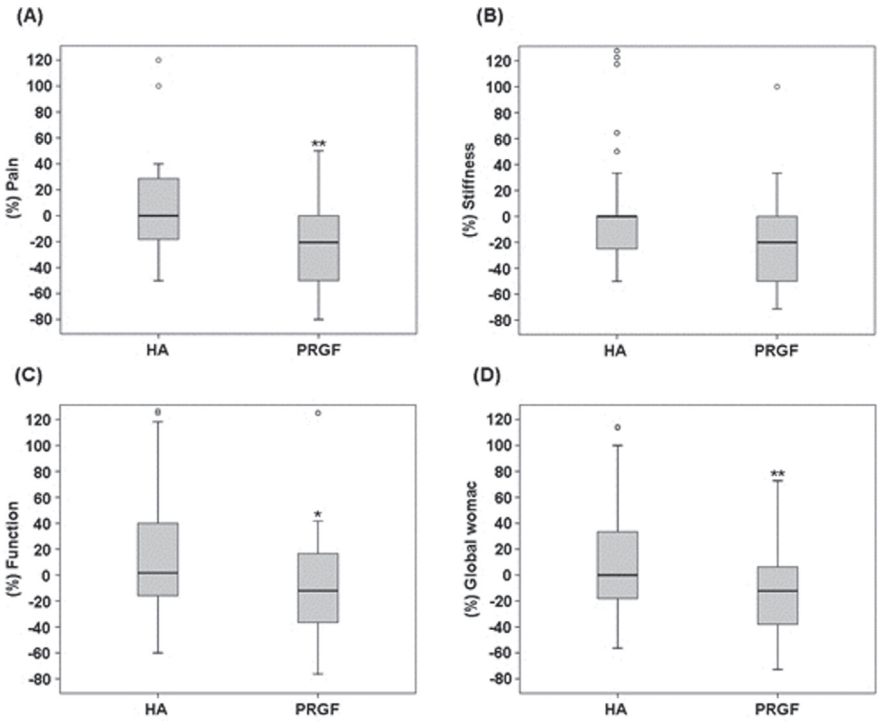

Fig. 1. Box and whiskers plot showing the percent reduction of WOMAC scales at five weeks post-treatment: (A) pain, (B) joint stiffness, (C) physical function and (D) global WOMAC score. Median, 25th to 75th centiles are shown.
$^{*}p<0.05$ and $^{**}p<0.01$ comparing to HA treatment.

Discussion

The use of autologous GFs has been proposed as a strategy for enhancing the cellular response to degenerative injury within the joint. The present study was undertaken to explore the feasibility and safety of the intra-articular injection of PRGF and to obtain initial useful information about the short-term clinical effects. The primary imperatives of a new therapy remain the control of symptoms. Since pain is the most pressing problem facing people with OA, a significant improvement in 33.3% of the patients injected with PRGF at 2 months indicates the potential of the proposed treatment. We also report on enhanced global efficacy of PRGF treatment *vs*. HA without any complication, although results need to be interpreted with caution owing to the small number of patients.
In the past decade, several crucial roles of GFs have been identified in joint repair. For example current understanding of TGF-β1 suggests that it is essential for cartilage integrity and a powerful tool to prevent or repair cartilage damage (13). If such a role is confirmed for platelet-derived TGF-β, intra-articular administration of PRGF could retard or prevent progression of degeneration of the joint. On the catabolic side, PRGF will also provide an exogenous source of TIMPs and α2-macroglobulin, the latter a natural endoproteinase inhibitor that can inhibit metalloproteinase activity in addition to preventing the breakdown of cartilage aggrecan by ADAMTS-4 and -5 (14).
Due to the localized nature of OA, the intra-articular administration of this unique molecular mixture along with its biocompatibility and non-inmunogenicity may be an attractive approach for OA treatment. In effect the autologous nature of this therapy is very relevant in OA management since the disease primarily affects people over the age of 60, who are most prone to drug toxicity. The risk of disease transmission or an antigenic reaction is non-existent because autologous blood is not mixed with exogenous thrombin or any other component of animal or human origin. From a safety standpoint, leukocytes have been eliminated since neutrophils express matrix degrading enzymes, such as matrix metalloproteinase-8 and -9 and release reactive oxygen species that destroy local cells (15). Additionally, it has been reported that platelets also store antibacterial and fungicidal proteins that could prevent infection (16).
Our retrospective study has several inherent weaknesses especially due to the incompleteness of data entered in the patients' records, for example mean disease duration or detailed consumption of analgesics was not documented. Although a randomized controlled pilot trial is imperative, in an attempt to partially overcome our limitations we have carefully matched patients in both groups for the most relevant correlates. Based on this preliminary study we suggest that knee OA treated with the application of autologous platelet-rich fibrin could present new possibilities for enhanced outcomes. Although these preliminary results need confirmation in a randomized clinical trial, they provide useful information about the safety of this new procedure and open new perspectives in the area of joint diseases.

References

1. SCHMIDT MB, CHEN EH, LYNCH SE: A review of the effects of insulin-like growth factor and platelet-derived growth factor on *in vivo* cartilage healing and repair. *Osteoarthritis Cartilage* 2005; 14: 403-12.
2. GELSE K, SCHNEIDER H: *Ex vivo* therapy approaches to cartilage repair. *Advanced Drug Delivery* 2006; 58: 259-84.
3. ANITUA E, ANDIA I, ARDANZA B, NURDEN P, NURDEN AT: Autologous platelets as a source for healing and tissue regeneration. *Thromb Haemost* 2004; 91: 4-15.
4. SÁNCHEZ M, AZOFRA J, ANITUA E *et al.*: Plasma rich in growth factors to treat an articular cartilage avulsion: a case report. *Med Sci Sports Exerc* 2003; 35: 1648-52.
5. SÁNCHEZ M, AZOFRA J, AIZPURÚA B, ELORRIAGA R, ANITUA E, ANDÍA I: [Use of autologous plasma rich in growth factors in Arthroscopic surgery] (Spanish). *Cuadernos de Artroscopia* 2003; 10: 12-19.
6. ANITUA E, SANCHEZ M, NURDEN AT, NURDEN P, ORIVE G, ANDIA I: New insights into and novel applications for platelet-rich

therapies. *Trends in Biotechnology* 2006; 24: 227-36.
7. SÁNCHEZ M, ANITUA E, AZOFRA J, ANDIA I, PADILLA S, MÚJICA I: Comparison of surgically repaired Achilles tendon tears using PRGF. *Am J Sports Med* 2007; 35: 245-51.
8. ANITUA E, AGUIRRE JJ, ALGORTA J *et al*.: Effectiveness of autologous preparation rich in growth factors for the treatment of chronic cutaneous ulcers. *J Biomed Mater Res B Appl Biomater* 2008; 84: 415-21.
9. ANITUA E, SÁNCHEZ M, NURDEN AT *et al*.: Platelet-released growth factors enhance the secretion of hyaluronic acid and induce hepatocyte growth factor production by synovial fibroblasts from arthritic patients. *Rheumatology* 2007; 46: 1769-72.
10. CARMONA JU, ARGÜELLES D, CLIMENT F, PRADES M: Autologous platelet concentrates as a treatment of horses with osteoarthritis: a preliminary clinical study. *J Equine Vet Sci* 2007; 27: 167-70.
11. ALTMAN R, ASCH E, BLOCH D *et al*.: Development of criteria for the classification and reporting of osteoarthritis: classification of osteoarthritis of the knee. *Arthritis Rheum* 1986; 29: 1039-49.
12. ESCOBAR A, QUINTANA JM, BILBAO A, AZKARATE J, GUENAGA JI: Validation of the Spanish version of the WOMAC questionnaire for patients with hip or knee osteoarthritis. Western Ontario and McMaster Universities Osteoarthritis Index. *Clin Rheumatol* 2002; 21: 466-71.
13. BLANEY DAVIDSON EN, VAN DER KRAAN PM, VAN DEN BERG WB: Review. TGF-β and osteoarthritis. *Osteoarthritis Cartilage* 2007; 15: 597-604.
14. TORTORELLA MD, ARNER EC, HILLS R *et al*.: Alpha2-macroglobulin is a novel substrate for ADAMTS-4 and ADAMTS-5 and represents an endogenous inhibitor of these enzymes. *J Biol Chem* 2004; 279: 17554-61.
15. BRAMONO DS, RICHMOND JC, WETZEL PP, KAPLAN DL, ALTMAN GF: Matrix metalloproteinases and their clinical applications in orthopaedics. *Clin Orthop Relat Res* 2004; 428: 272-85.
16. BIELECKI TM, GAZDZIK TS, ARENDT J, SZCZEPAUSKI T, KROL W, WIELKOSZYNSKIT T: Antibacterial effect of autologous platelet gel enriched with growth factors and other active substances. *In vitro* study. *J Bone Joint Surg Br* 2007; 89: 417-20.