Growth factors in the treatment of early osteoarthritis

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Summary

Regenerative medicine is the science that studies the regeneration of biological tissues obtained through use of cells, with the aid of support structures and with biomolecules such as growth factors.
As regards the growth factors the PRP, or the platelet-rich plasma, obtained from a withdrawal of autologous blood, concentrating the platelets, represents a safe, economical, easy to prepare and easy to apply source of growth factors.
Numerous growth factors are in fact within the platelets and in particular a large number of them have a specific activity on neoproliferation, on cartilage regeneration and in particular also an antiapoptotic effect on chondroblasts:
- **The PDGF which regulates the secretion and synthesis of collagen;**
- **The EGF that causes cellular proliferation, endothelial chemotaxis and angiogenesis;**
- **The VEGF that increases angiogenesis and vascular permeability;**
- **The TGF-beta that stimulates the proliferation of undifferentiated MSC, stimulates chemotaxis of endothelial cells and angiogenesis;**
- **The bFGF that promotes the growth and differentiation of chondrocytes and osteoblasts stimulates mitogenesis of mesenchymal cells, chondrocytes and osteoblasts.**

These properties have led to the development of studies that evaluated the efficacy of treatment of infiltrations in the knee and hip with platelet-derived growth factors.
Regarding the knee it was demonstrated that in patients with moderate degree of gonarthrosis, the PRP is able to significantly reduce the pain and improve joint function, both on placebo and towards infiltrations with hyaluronic acid.
The success of the treatment was proportional to the age of and inversely proportional to the severity of osteoarthritis according to Kellgren and Lawrence classification.
The possibility of infiltrations guided with ultrasound into the hip led us to extend the indications also to hip arthrosis, as already showed by Sanchez.
Even in coxarthrosis preliminary results at 6 and 12 months show that a cycle of 3 infiltrations of PRP has significantly decreased the pain and increased range of motion and joint function.

KEY WORDS: growth factors; platelet rich plasma; early osteoarthritis.

Introduction

Osteoarthritis is a prevalent and disabling disease affecting an increasingly larger percentage of world population. While clinical osteoarthritis (OA) is a late-stage condition for which disease-modifying opportunities are limited, osteoarthritis typically develops over decades, offering a long window of time to potentially alter its course. So there is a large population of young and active people with early osteoarthritis who have not yet the classical signs of OA but who are strong candidates to an increased risk for accelerated development of joint degeneration. It is a population who need newer strategies for the development of disease-modifying treatments (1).
Many options have been made available to address problems regarding cartilage damage, each with its own advantages and disadvantages. Growth factors could have a crucial role in this process since they influence chemotaxis, differentiation, proliferation and synthetic activity of cartilage and bone cells, thereby regulating physiological remodeling and cartilage healing. That makes the use of the autologous and recombinant growth factors (GF) a rapidly growing field of orthopedics focusing on manipulating GF and secretory proteins to maximize the healing of cartilage, soft tissues and bone (2).
A variety of growth factors have been found to play a role in bone healing, but the two most important families can be classified as: Bone-Derived Growth Factors, namely the BMPs family, which are mainly used for bone regeneration, and the Autologous Blood-Derived Growth Factors, which are dedicated to cartilage and soft tissue regeneration.
Most of the growth factors derived from autologous blood are released upon platelet activation, and their clinical use has been popularized with Platelet-rich plasma (PRP); Platelet-rich plasma is obtained from patients' blood using commercially available devices and it could release growth factors for the enhancement of cartilage and soft tissue healing as demonstrated by basic science and clinical studies.
The autologus nature of PRP, its ease of application and relative low cost are some of the advantages of PRP that have led to research interest and to a wide clinical application (3).
The purpose of this paper, therefore, is to provide back-ground on the underlying basic science, the methods used for producing PRP and an overview of evidence based medicine on clinical application of PRP in the treatment of early osteoarthritis.

Preclinical and clinical studies

With increasing frequency, platelet-rich plasma (PRP) preparations have been used to treat cartilage lesions to regenerate tissue homeostasis and to delay the progression of knee osteoarthritis (OA). Growth factors are obvious tools to enhance cartilage repair and platelets are rich in these factors. The rationale is based on the activity of blood growth factors. The growth factors are a diverse group of polypeptides that have important roles in the regulation of growth and tissue development, determining the behaviour of all cells, including chondrocytes. To understand how they influence cartilage repair is the key to an increasing use of these factors in such problems and understanding the reactivities in normal and arthritic cartilage and potential side effects on other compartments in the joint will help to identify possibilities and limitations. Chondrocytes are affected by numerous extracellular stimuli influencing the regulation of biosynthetic and catabolic activity, including mechanical stress and soluble factors (4). An imbalance of regulatory factors, which may result from ageing, disease, or injury, may hinder tissue maintenance and repair, ultimately resulting in deleterious changes in gene expression, altered extracellular matrix, tissue degeneration and consequently an accelerated erosion of the articular surface, leading to end-stage arthritis (5).

The properties of PRP are based on the production and release of multiple growth and differentiation factors. The basic cytokines identified in platelets regarding cartilage regeneration include:

PDGF (Platelet-derived Growth Factor) appears to be the first growth factor present in a wound and initiates connective tissue healing through the promotion of collagen and protein synthesis.

The primary effect of PDGF seems to be its mitogenic activity to mesoderm-derived cells such as fibroblasts, vascular muscle cells, glial cells and condrocytes. The most important specific activities of PDGF include angiogenesis and chemotaxis for fibroblasts and collagen synthesis.

Evidence to support the use of PDGF in cartilage repair is extrapolated from the role of PDGF in wound healing or stimulation of matrix synthesis in growth plate chondrocytes (6). *In vivo*, when injected into the knee of skeletally immature rats, no adverse effects were noted in the cartilage or synovial membrane (7).

TGF- β (Transforming Growth Factor β) superfamily are structurally related and only active as homo-or heterodimers linked together with a single disulfide bond (8). More recently, *in vitro* studies of cartilage-derived morphogenetic protein (CDMP-1) (also known as GDF-5; growth differentiation factor-5) and CDMP-2 have been performed and all of them show some capacity to stimulate cartilage matrix synthesis. TGF-b3 also stimulated extracellular matrix (ECM) synthesis and has been evaluated *in vitro* in rabbit models of acute cartilage injury (9-11). TGF-b1 stimulates chondrocyte synthetic activity and decreases the catabolic activity of IL-1 (12). *In vitro* TGF-b1 stimulates chondrogenesis of synovial lining and bone marrow-derived MSCs (13, 14). There have also been promising studies in rabbits in which TGF-b1 enhanced repair of cartilage defects (15).

FGF is another family of factors which has been shown to have positive effect on cartilage repair that have been studied. In cartilage, FGF-2 (also known as basic FGF [bFGF]) is found in relative abundance in the pericellular matrix of cartilage (16). On loading, FGF-2 becomes bound to cell surface receptors and activates anabolic pathways leading to decreased aggrecanase activity but no apparent change in proteoglycan content occurs.

VEGF (Vascular Endothelial Growth Factor) is the major regulator of vasculogenesis and angiogenesis and playing an important role in tissue regeneration (17).

CTGF (Connective tissue growth factor). This new Growth Factor was described very recently by Kubota and others; Platelets adhere to CTGF at injured tissue wound sites, where it is overexpressed along with the platelet coagulation process. In their experiments they showed that non-activated platelets contain considerable amounts of CTGF and that is released by activated PRP, endorsing angiogenetic activity and cartilage regeneration (3).

Many animal studies assessed the PRP effect on chondrogenesis and cartilage healing:

Blood-derived GFs have already been studied for their potential in helping cartilage repair (18-25). Gaissmaier et al. investigated the effect of human platelets supernatant on chondrocytes in human articular biopsy specimens and observed an acceleration of chondrocyte expansion (26).

Mishra et al. (27) tested whether PRP may be useful specifically for cartilage regeneration, and a cell culture experiment was devised in which mesenchymal stem cells (MSCs) were grown in control media or media enhanced with inactivated, buffered PRP: their results demonstrated that PRP enhances MSC proliferation and suggest that PRP causes chondrogenic differentiation of MSC *in vitro.*

More recently in a rabbit model, 48 osteochondral defects were divided into three groups: no treatment, treatment with autogenous PRP in poly-lacticglycolic acid, and with poly-lacticglycolic acid alone. The PRP treatment group demonstrated a greater extent of cartilage regeneration, as well as higher production of glycosaminoglycans in the extracellular matrix (28).

The degree of cartilage regeneration is also influenced by the severity of OA. Kwon et al. (2012) in a rabbit model demonstrated that intra-articular PRP injection influences cartilage regeneration in all severities of rabbit knee OA, but the cartilage regenerative power of PRP injection in moderate knee OA was greater than that in mild or very mild OA (29).

These studies suggest an important role for these potent biologic regulators of chondrocytes in cartilage repair, but there is always a paucity of human clinical studies about cartilage repair with the use of PRP.

One of the first studies was performed by Sanchez et al. (2008) with an observational retrospective cohort study using hyaluronan injections as a control; at final follow up there were better results in the group treated with intra-articular injections of an autologous preparation rich in growth factors (30).

Kon et al. (2011) in a study that compared injection in early degenerative knee arthrosis with HA versus PRP demonstrated that autologous PRP injections could provide more and longer efficacy than HA injections in reducing pain and symptoms and recovering articular function. Better results were achieved in younger patients with a lower degree of cartilage degeneration (31).

Sampson et al. in a perspective, uncontrolled study, administered 3 PRP injections at 4-week intervals to 14 patients with knee OA. Significant improvements were found, with relief of pain and symptoms (32). Wang-Saegusa et al. in a second perspective, nonrandomized, longitudinal study, 261 patients with knee OA (Outerbridge grades I-IV) were given 3 intra-articular injections of platelet concentrate suspended in plasma from autologous blood at 2-week intervals. Participants had statistically significant improvements in pain and function at 6 months (33).

Filardo et al. in a third case series administered PRP injections in patients with knee OA. At the 2-year follow-up, patients had diminished gains compared with their results at the 1-year follow-up, although outcomes remained better than baseline levels (34).

Spakova et al. in their study aimed to find a simple, cost-effective, and time-efficient method for the preparation of platelet-rich plasma (PRP), so the acquired benefits will be readily available for multiple procedures in smaller outpatient clinics and to explore the safety and efficacy of the application of PRP in the treatment of degenerative lesions of articular cartilage of

the knee. They recorded statistically significantly better results in the Western Ontario and McMaster Universities Osteoarthritis Index and Numeric Rating Scale scores in a group of patients who received PRP injections after a 3- and 6-months follow-up so they support the application of autologous PRP as an effective and safe method in the treatment of the initial stages of knee osteoarthritis (35).
Fewer studies have been performed in hip OA, but with similar results. Sanchez et al. treated 40 patients with ultrasound-guided platelet-rich plasma injections for the treatment of osteoarthritis of the hip. At final follow-up statistically significant reductions in VAS, WOMAC and Harris hip subscores for pain and function were reported ($P<0.05$) (36).

Conclusion and future directions

In conclusion platelet rich plasma is a safe, autologous, easy to prepare and to use and relative low cost procedure to deliver growth factors for cartilage healing and regeneration.
The clinical results of comparative studies suggest that this procedure may be useful for the treatment of degenerative articular pathology of the knee as a treatment for articular cartilage degeneration in humans, in which produces a significant improvement in the clinical outcome of most patients especially in the short term. Studies support efficacy in terms of antiapoptotic activity of PRP and repair in cartilage lesions. Treatment with this procedure of early degenerative arthrosis seems to be promising because of the interest and for the economical burden in term of disability that this problem entails. Lack of randomized controlled study limits at the moment a conclusive statement on the efficacy of this procedure and it still remains to be determined whether there is only a temporary improvement in symptoms or whether PRP therapy may play a more important role through disease-modifying therapy.
Furthermore recent studies support the use of PRP intra-articular injections, not only as a conservative treatment, but also after surgical procedures as many researchers attempted to enhance cartilage repair by combining surgical procedures such as microfractures with growth factors (GFs). PRP has been shown to improve the reparative response of focal defects of articular cartilage after surgery compared to surgery alone and also to increase the durability of repair tissue over time (37).
Many clinical questions still have to be clarified, particularly with regard to the timing of therapy, the volume and frequency of treatment and the ideal composition of the platelet-rich plasma. However, because the majority of the clinical trials have shown encouraging outcomes, further controlled clinical trials will help to elucidate the effects of platelet rich plasma on cartilage regeneration.

References

1. Chu CR, Williams AA, Coyle CH, et al. Early diagnosis to enable early treatment of pre-osteoarthritis. Arthritis research & therapy 2012 Jun;14(3):212.
2. Chung R, Foster BK, Xian CJ. Preclinical Studies on Mesenchymal Stem Cell-Based Therapy for Growth Plate Cartilage Injury Repair. Stem Cells Int 2011;2011:570125.
3. Civinini R, Macera A, Nistri L, et al. The use of autologous blood-derived growth factors in bone regeneration Clin Cases Miner Bone Metab 2011 Jan-Apr;8(1):25-31.
4. Bucholz RW, Einhorn TA, Marsh JL. Bone and joint healing. In: Bucholz RW, Heckman JD, Court-Brown C; Sixth ed. Lippincott Williams & Wilkins; 2006. pp. 300-11.
5. Alves H, Munoz-Najar J, de Wit JR, et al. A link between the accumulation of DNA damage and loss of multipotency of human mesenchymal stromal cell. J Cell Mol Med 2010;14:2729-2738.
6. Fortier LA, Barker JU, Strauss EJ, et al. The Role of Growth Factors in Cartilage Repair Clin Orthop Relat Res 2011 October;469(10):2706-2715.
7. Kwon DR, Park GJ. Intra-Articular Injections for the Treatment of Osteoarthritis: Focus on the Clinical Use of Several Regimens. Osteoarthritis - Diagnosis, Treatment and Surgery 67-100.
8. Wu MY, Hill CS. TGF-beta superfamily signaling in embryonic development and homeostasis. Dev Cell 2009;16:329-343.
9. Ellman MB, An HS, Muddasani P, et al., Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene 2008;420:82-89.
10. Kurth T, Hedbom E, Shintani N, et al. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. Osteoarthritis Cartilage 2007;15:1178-1189.
11. Lee CH, Cook JL, Mendelson A, et al. Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study. Lancet 2010;376:440-448.
12. Lotz M, Rosen F, McCabe G, et al. Interleukin 1suppresses transforming growth factor-induced inorganic pyrophosphate (PPi) production and expression of the PPi-generating enzyme PC-1 in human chondrocytes. Proc Natl Acad Sci USA 1995 October;Vol. 92:pp. 10364-10368.
13. Fan J, Gong Y, Ren L, et al. In vitro engineered cartilage using synovium derived mesenchymal stem cells with injectable gellan hydrogels. Acta Biomater 2010;6:1178-1185.
14. Kurth T, Hedbom E, Shintani N, et al. Chondrogenic potential of human synovial mesenchymal stem cells in alginate. Osteoarthritis Cartilage 2007;15:1178-1189.
15. Diao H, Wang J, Shen C, et al. Improved cartilage regeneration utilizing mesenchymal stem cells in TGF-beta1 gene-activated scaffolds. Tissue Eng Part A 2009;15:2687-2698.
16. Ellman MB, An HS, Muddasani P, et al. Biological impact of the fibroblast growth factor family on articular cartilage and intervertebral disc homeostasis. Gene 2008;420:82-89.
17. Hoeben A, Landuyt B, Highley MS, et al. Vascular Endothelial Growth Factor and Angiogenesis. Pharmacological Reviews 2004 December;vol. 56, no. 4:549-580.
18. Andrew JG, Hoyland JA, Freemont AJ, et al. Platelet-derived growth factor expression in normally healing human fractures. Bone 1995;16(4):455-460.
19. Marx RE. Platelet-rich plasma: evidence to support its use. J Oral Maxillofac Surg 2004;62:498-93.
20. Tayapongsak P, O'Brien DA, Monteiro CB, et al. Autologous fibrin adhesive in mandibular reconstruction with particulate cancellous bone and marrow. J Oral Maxillofac Surg 1994;52:161-5.
21. Wiltfang J, Kloss FR, Kessler P, et al. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in criticalsize defects. Clin Oral Implants Res 2004;15(2):187-93.
22. Al Sukhun J, Helenius M, Lindqvist C, et al. Use of platelet rich plasma (PRP) in the reconstruction of mandibular bony defects: clinical and radiographic follow-up. Br J Oral Maxillofac Surg 2007 Jan 6.
23. Dugrillon A, Klüter H. Topical application of platelets for improved wound healing. Blood Ther Med 2002;3(1):21-6.
24. Thor A, Franke-Stenport V, Johansson CB, et al. Early bone formation in human bone grafts treated with plateletrich plasma: Preliminary histomorphometric results. Int. J Oral Maxillofac Surg 2007;36:1164-1171.
25. Ranly DM, Lohmann CH, Andreacchio D, et al. Platelet-rich plasma inhibits demineralized bone matrix-induced bone formation in nude mice. J Bone Joint Surg Am 2007;89(1):139-47.
26. Gaissmaier C, Koh JL, Weise K. Growth and differentiation factors for cartilage healing and repair. Injury 2008;39:S88-S96.
27. Mishra A, Tummala P, King A, et al. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng Part C Methods 2009 Sep;15(3):431-5.
28. Sun Y, Feng Y, Zhang CQ, et al. The regenerative effect of platelet-rich plasma on healing in large osteochondral defects. Int Orthop 2010 Apr;34(4):589-97.
29. Kwon DR, Park GY, Lee SU. The effects of intra-articular platelet-rich plasma injection according to the severity of collagenase-induced knee osteoarthritis in a rabbit model. Ann Rehabil Med 2012 Aug;36(4):458-65.
30. Sanchez M, Anitua E, Azofra J, et al. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. Clin Exp Rheumatol 2008 Sep-Oct;26(5):910-3.

31. Kon E, Mandelbaum B, Buda R, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. Arthroscopy 2011 Nov;27(11):1490-501.
32. Sampson S, Reed M, Silvers H, et al. Injection of platelet-rich plasma in patients with primary and secondary knee osteoarthritis: a pilot study. Am J Phys Med Rehabil 2010 Dec;89(12):961-9.
33. Wang-Saegusa A, Cugat R, Ares O, et al. Infiltration of plasma rich in growth factors for osteoarthritis of the knee short-term effeects on function and quality of life. Arch Orthop Trauma Surg 2011;131:311-317.
34. Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2011 Apr;19(4):528-35.
35. Spakova T, Rosocha J, Lacko M, et al. Treatment of Knee Joint Osteoarthritis with Autologous Platelet-Rich Plasma in Comparison with Hyaluronic Acid. American Journal of Physical Medicine & Rehabilitation 2012 May; 91(5):411-417.
36. Sanchez M., Guadilla J, Fiz N, et al. Ultrasound-guided platelet-rich plasma injections for the treatment of osteoarthritis of the hip. Rheumatology 2012;51:144-150.
37. Milano G, Deriu L, Sanna Passino E, et al. Repeated platelet concentrate injections enhance reparative response of microfractures in the treatment of chondral defects of the knee: an experimental study in an animal model. Arthroscopy 2012 May;28(5):688-701.