PLATELET RICH PLASMA AND TENDINOPATHY: STATE OF THE ART

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Platelet-rich plasma (PRP) is increasingly used in the management of tendon injury in sports, supposedly accelerating the process of healing, tissue regeneration, and return to play. However, the scientific clinical evidence to support its use is scanty, and more level I studies need to be performed to justify its widespread use.

Tendon problems account for 30-50% of all sports lesions occurring in professional and recreational athletes[1]. Tendon injuries are sport-specific. Achilles tendinopathy is more prevalent in runners, while rotator cuff problems in overhead and high-force athletes. Platelet-rich plasma (PRP) is increasingly used in sports medicine, because supposedly accelerating the process of healing, tissue regeneration, and return to play, particularly in elite and professional athletes. Firstly used in the 1980s to promote physiological wound healing of cutaneous ulcers[2], PRP has potential regenerative and healing effects in oral implantology[3]. Successively, the use of PRP has spread to other clinical areas, including ophthalmology, orthopaedics, sports medicine, cardiology, dermatology, plastic surgery, and neurology. Concerning musculoskeletal disorders, PRP has been used to enhance the healing of meniscus defects and muscle injuries, stimulate chondrocytes to engineer cartilaginous tissue, reduce pain and produce better and more balanced synovial fluid in arthritic knees, improve outcomes after total knee arthroplasty and subacromial decompression, accelerate bone formation, stimulate the healing of anterior cruciate ligament injury central defects, its primary repair or its reconstruction, improve the outcome of operated ruptured Achilles tendons, reduce pain in chronic tendinopathies, and prevent and reverse inter-vertebral disc degeneration[4].

The present review aims to describe the status of the field of PR therapies and question whether this knowledge can be applied for clinical benefit in patients with tendinopathy. Finally, we summarize the different hypotheses regarding the biological mechanisms underlying tendinopathy.

PLATELET-RICH PLASMA THERAPIES AND HEALING MECHANISMS

Produced in large numbers from megakaryocytes in the bone marrow, activated platelets secrete multiple signaling proteins involved in the healing of musculoskeletal tissues, such as transforming growth factor-β1 (TGF-β1), platelet-derived growth factor (PDGF)-AB and PDGF-BB, vascular endothelial growth factor (VEGF-A), epithelial growth factor (EGF), hepatocyte growth factor (HGF) and insulin-like growth factor (IGF-I and IGF-II)[5]. By interacting with membrane receptors, growth factors activate various intracellular signaling pathways inducing angiogenesis or extracellular matrix formation, crucial in the process of repair of tendons, muscles, ligaments, cartilage, and bone injuries. After muscle strain or contusion, the rationale for PRP therapy lies in reversing the blood ratio by decreasing red blood cell (RBC) amount, which are less useful in the healing process, to approximately 5%, and increasing the platelet amount to 94% to stimulate recovery[6]. These growth factors and further released growth factors supposedly improve the healing process in chronic injuries, and accelerate repair in acute lesions[6].

Key words:

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0394-6320 (2011)

PREPARATION OF PRP, PRODUCTS, AND PRODUCT SAFETY

The process of PRP preparation is relatively rapid and straightforward, and can be undertaken in the clinic or the operating room. Costs vary tremendously: a commercial kit yields a PRP concentrate at the cost of several hundred pound, but in house non-automatised techniques can produce a PRP concentrate for approximately US$10[6]. For PRP preparation, peripheral blood is drawn from the patient, with or without anticoagulants, and the plasma is centrifuged or filtrated. On the basis of the size and phase of injury, different volumes, varying from 10 to 100 mL, may be applied. To determine the composition and concentration in terms of leukocytes, erythrocytes, and platelets in a given plasma volume, there are 3 methods of preparing PRP : 1) double-spinning methods using automated machines along with commercial kits, 2) single-spinning methods using conventional laboratory centrifuges followed by manual PRP separation, or 3) selective blood filtration using commercial available technology[7]. When using single spinning, the platelet yield is 1- to 3-fold baseline levels, while 5- to 8-fold baseline levels are achieved by double spinning. Double spinning also concentrates leukocytes. There are pure PRP (P-PRP), in which leukocytes are purposely eliminated from the PRP, and leukocyte and platelet-rich plasma (L-PRP), which contains a high concentration of leukocytes[7]. At present, the role of leukocytes, in particular in orthopedic sports medicine applications, is controversial[8]. However, the improved P-PRP homogeneity and its reduced donor-to donor variability allow to define some PRP production techniques more reproducible and predictable than others. Double spinning techniques provide a PRP concentrate of around 10% of the blood volume drawn (i.e. 20 mL of whole blood would result in 2 mL of PRP), in contrast to 40% to 50% of the blood volume obtained after single spinning. These differently obtained products present varying biological properties and potential uses, but it is unclear whether their use produces clinically relevant differences[8]. It has been suggested that PRP preparations containing only moderately elevated platelet concentrations induce optimal biological benefit, lower platelet concentrations produce suboptimal effects, and higher ones produce inhibitory effects[9]. Age may influence the number of receptors of local cells interacting with the plasma signals[10]. The plasma should be prepared and immediately used at the point of care, should not be stored and, prior to application, platelets can be slowly activated with the addition of calcium chloride, a necessary cofactor for prothrombin conversion into thrombin or adding standard solution of 1000 U/mL of bovine or human thrombin along with 10% calcium chloride. Once plasma has been activated, the fibrin scaffold can be formed in vivo or ex vivo to gradually release growth factors[10].

Given the autologous nature of PRP, the possible transmission of diseases such as human immunodeficiency virus, hepatitis, or Creutzfeldt- Jakob disease, or of immunogenic reactions (a concern with allografts or xenografts), is of concern[11]. Some systems using purified bovine thrombin to activate the platelets have been hypothesized to produce coagulopathies. Therefore, human recombinant thrombin are now being advocated. The genetic instability and the possible role of PRP in the development of neoplasms have been also addressed. Growth factors act on receptors located on the cell membranes, activating normal gene expression[3], with no direct mutagenic effect. However, until now, no systemic effect on circulating growth factors has been shown after PRP application[12]. The in vitro and in vivo antimicrobial activity of PRP (platelet-leukocyte gel) against Staphylococcus aureus is not comparable with systemic antibiotic treatments[12].

PRP therapies are interestingly applied to manage tendon injuries. Associated with a failed healing response, tendinopathy is typically accompanied by increased turnover and remodeling, and gradual transformation of extracellular matrix. Tenoblasts and tenocytes are involved to repair and maintain the extracellular matrix, influenced by external growth factors and cytokines released from PRPs[13]. PRPs has chemotactic action[14], stimulates cell proliferation and the synthesis of angiogenic factors[15, 16], the synthesis of molecules of the extracellular matrix[16]. In a study on 12 athletes undergoing surgical Achilles tendon repair, Sanchez et al[17] applied P-PRP with a moderate concentration of platelets (2–3 times the concentration of platelets compared with whole blood) clotted ex vivo, while controls received an identical surgical procedure with no PRP administration. Enhanced range of motion and faster return to sporting activities were reported by the group of patients who had received PRP during surgery. The cross-sectional area of these Achilles tendons had less differences compared with the contra-lateral tendon after 18 months, indicating a more physiological repair of the PRP-treated tendon. Recently, buffered L-PRP injection did not improve pain or activity of patients with Achilles tendinopathy concurrently undergoing eccentric exercise regimen[18]. Only 1 injection of L-PRP was performed, but most benefits in Achilles tendinopathy and tendinopathies in general have been reported after 2 to 3 injections. Different clinical effects of PRP have been observed, with favorable preliminary studies in patients managed for wrist extensor and flexor tendinopathy. Significantly improved pain has been observed after 8 weeks from buffered L-platelet concentrate injections[19]. More recently, in a randomized clinical trial[20] reporting

on patients with chronic tennis elbow tendinopathy, the group treated with corticosteroids recovered initially and declined successively, whereas the L-PRP group progressively improved. PRP application provided significant functional improvement 12 and 24 months after arthroscopic rotator cuff repair, and in athletes with chronic patellar tendinopathy (jumper's knee). In a prospective case-control study, activated L-PRP was effective in patellar tendinopathy at 6 month follow-up. Concerning the effects of PRP on rotator cuff pathology, Everts et al[21] reported better functional recovery and less pain in patients undergoing L-PRP injections after open subacromial decompression, with no group differences at 2 years. Recently, Castricini et al reported that PRP[22] applied during rotator cuff surgery had the same outcomes as controls.

PLATELET-RICH THERAPIES AND TENDINOPATHY

When tendons are injured, immune, vascular and nervous cells, resident or migratory fibroblasts and tendon progenitor cells sense environmental changes, and try to reverse the stressful alterations by initiating a healing cascade that results in complex biochemical changes[13]. PRP therapies include all platelet-rich plasma, technologies and re-administration procedures. Activated at the site of tissue injury, platelets release intracellular stores, predominantly a-granules, dense granules and lysosomes[23]. Even though the best effectors of PRP therapies are growth factors such as PDGF, TGF, FGF, endothelial growth factor (EGF), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF) and VEGF, this process seems to be more complex, because a-granules contain more than 300 proteins[23]. In addition, PRP therapies contain structural proteins (e.g., fibrin, fibronectin and vitronectin) which facilitate cell adhesion by forming three-dimensional scaffolds. The term tendinopathy refers to the pain and swelling of a tendon, associated with the histopathological findings of intratendinous healing failure, occurring when tissue breakdown exceeds the rate of tissue healing or the capacity for tissue repair is impaired[24]. Currently, cell apoptosis, deregulated angiogenesis or pain and inflammation have been hypothesized to be involved, with no mutual exclusion, but simultaneously occurring at different temporal stages[24].

Apoptosis Cell proliferation and cell death are finely balanced by intrinsic or extrinsic factors, whose imbalance may result in excessive cell loss and tendon tissue disruption. An extrinsic cause of apoptosis is the relative hypovascularity in the midportion of the Achilles tendon or supraspinatus[24], related to an exercise-induced hyperthermia. Another extrinsic factor may be the loss of homeostatic tension from microscopic collagen breakdown during demanding exercise or overuse[25]. Indeed, tendon cells lacking appropriate extracellular matrix (ECM) attachment are rapidly eliminated by means of apoptosis. Intrinsic pathway of apoptosis (Bcl-2- inhibitable or mitochondrial) are used to respond to various intracellular stresses, such as DNA damage, unfolding stress in the endoplasmic reticulum, and death receptor stimulation[25]. Chemotactic factors can be released to attract phagocytes towards sites of apoptotic cell death, eliciting the release of lactoferrin, an anti-inflammatory glucoprotein that prevents any aberrant inflammation at sites of physiological cell death. One study has shown a large number of apoptotic cells in ruptured supraspinatus tendons[26], and other studies showed excessive apoptosis in tendinopathic patellar tendon specimens in athletes[27] and non-insertional Achilles tendinopathy[28]. In degenerative human supraspinatus tissues, down regulation anti-apoptotic heat shock proteins (HSP27 and HSP70) and up-regulation of pro-apoptotic genes such as caspase-3 and -8 and FADD-like IL-1b converting enzyme (FLICE) inhibitory protein[29]. At present IGF-I[30], Cartilage oligomeric protein (COMP) seem to be protective cells against death, by elevating members of the inhibitors of apoptosis (IAP) family of survival proteins[30]. However, further anti-apoptotic potential of plasma preparations should be discovered to improve our knowledge in this field.

Deregulated angiogenesis At histopathology, fibroblastic cellularity and haphazard neovascularization are indicative of failed healing response[31]. VEGF participates in neo vessel growth, not in neovessel stabilization, and angiofibroblastic features may occur in tendinopathy[31]. In painful tendinopathy with high blood flow and prominent perivascular sympathetic innervation in the paratenon of the patellar and Achilles tendon, sclerosing injections in the point where the blood supply enters the tendon may reduce pain, probably interfering with the local nerve supply[32]. Glyceril trinitrate patches, delivering NO, increase blood vessel diameter, flow, with pain reduction and increased tendon strength and mobility[32].

Inflammation Prolonged repetitive mechanical loading increased the production of proinflammatory agents (e.g., cytokines such as IL-1b and TNF-a, protaglandins such as PGE2, and neuropeptides such as substance P and CTGF[33], and mast cells, mostly in the patellar tendons of patients with pain and swelling. Higher levels of PGE2 and COX-2 were also detected compared to controls. VEGF production and enhanced production of MMP-1, MMP-3 and MMP-13[33], both of which cause matrix destruction. However, ultrasound guided peritendinous injections of Adalimumab (a TNF blocker)

or Anakinra (an IL blocker) in Achilles tendinopathy had only a modest clinical effect in a small trial[34].

DISCUSSION

Rapid pain relief, tendon healing, and developing conservative treatments are advocated in athletes complaining of tendinopathy. It is difficult to control healing, and tendon healing and PRP therapies are of concern, with inability to define the best platelet concentration and methods of preparation[6]. The best PRP formulation for musculoskeletal injuries can be reached after the relations between PRP components, healing mechanisms, and functional outcome will be clarified. To standardize PRP formulations and procedures for application, differences between pure platelet-rich plasma and leukocyte-platelet concentrates, regarding tissue damage exacerbation, need to be established[8], as well as the optimal balance between plasma myogenic factors (IGFs and HGF), and platelet-secreted angiogenic or chemotactic factors[35]. The best timing for application is not clear, and the implications of physicochemical temporal conditions of the tissue (i.e., pH, NO and oxygen levels) should be evaluated[13]. Concerning the treatment effects, it is also mandatory to report and monitor complications.

No systemic effects have been evidenced after local PRP injection, but infections, further injuries and possible systemic effects related to autologous growth factors administration should be monitored. No scientific reports suggest potential cause-effect relationships between growth factors present in PRP and carcinogenesis[36]. In 2010, PRP was specifically mentioned in the WADA prohibited list for the first time, but the different PRP formulations and treatment methodologies have not been found to increase muscle growth beyond return to a normal physiological state, and the use of PRP injections for therapeutic purposes only does not violate the spirit of sport. Even though PRP has been removed in the 2011 Prohibited List, WADA will continue to review PRP use as new medical and scientific information becomes available[36]. Although the management of sports injuries with PRP injections has been advocated since 2003, this strategy has been mostly supported by scarce level III-IV studies. Some trials report a great positive effect[20], but more recent well executed and scientifically stricter ones report at best no effect[18, 22], and possibly detrimental effects of PRP[37].

Given our rudimentary knowledge of the mechanism of action of the PRPs, it is challenging to use this technology to promote early healing, and produce improved and accelerated functional recovery. We prompt researchers to undertake appropriately powered level I studies with adequate and relevant outcome measures and clinically appropriate follow up. Because of the relatively safety of these products, basic science, clinical discovery and patient-oriented research should be interdependent rather than successive steps. The substantial challenges of incorporating such research into clinical care must be pursued if the potential of PRPs is to be realized.

REFERENCES

1 Kannus P, Natri A. Etiology and pathophysiology of tendon ruptures in sports. Scand J Med Sci Sports 1997;7: 107-12.

2 Margolis DJ, Kantor J, Santanna J, Strom BL, Berlin JA. Effectiveness of platelet releasate for the treatment of diabetic neuropathic foot ulcers. Diabetes Care 2001;24: 483-8.

3 Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent 2001;10:225-8.

4 Mei-Dan O, Lippi G, Sanchez M, Andia I, Maffulli N. Autologous platelet-rich plasma: a revolution in soft tissue sports injury management? Phys Sportsmed 2005;38:127-35.

5 Nurden AT, Nurden P, Sanchez M, Andia I, Anitua E. Platelets and wound healing. Front Biosci 2008;13:3532-48.

6 Mei-Dan O, Mann G, Maffulli N. Platelet-rich plasma: any substance into it? Br J Sports Med 2001;44:618-9.

7 Dohan Ehrenfest DM, Rasmusson L, Albrektsson T. Classification of platelet concentrates: from pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). Trends Biotechnol 2009;27:158-67.

8 Tidball JG. Inflammatory processes in muscle injury and repair. Am J Physiol Regul Integr Comp Physiol 2005;288: R345-53.

9 Graziani F, Ivanovski S, Cei S, Ducci F, Tonetti M, Gabriele M. The in vitro effect of different PRP concentrations on osteoblasts and fibroblasts. Clin Oral Implants Res 2006;17:212-9.

10 Vavken P, Saad FA, Murray MM. Age dependence of expression of growth factor receptors in porcine ACL fibroblasts. J Orthop Res2006;28:1107-12.

11 Landesberg R, Moses M, Karpatkin M. Risks of using platelet rich plasma gel. J Oral Maxillofac Surg 1998;56: 1116-7.

12 Banfi G, Corsi MM, Volpi P. Could platelet rich plasma have effects on systemic circulating growth factors and cytokine release in orthopaedic applications? Br J Sports Med 2006;40:816.

13 Andia I, Sanchez M, Maffulli N. Platelet rich plasma therapies for sports muscle injuries: any evidence behind clinical practice? Expert Opin Biol Ther **??????????**

14 Kajikawa Y, Morihara T, Sakamoto H, Matsuda K,

Oshima Y, Yoshida A, Nagae M, Arai Y, Kawata M, Kubo T. Platelet-rich plasma enhances the initial mobilization of circulation-derived cells for tendon healing. J Cell Physiol 2008;215:837-45.

15 Anitua E, Andia I, Sanchez M, Azofra J, del Mar Zalduendo M, de la Fuente M, Nurden P, Nurden AT. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF production by human tendon cells in culture. J Orthop Res 2005;23:281-6.

16 Anitua E, Sanchez M, Zalduendo MM, de la Fuente M, Prado R, Orive G, Andia I. Fibroblastic response to treatment with different preparations rich in growth factors. Cell Prolif 2009;42:162-70.

17 Sanchez M, Anitua E, Azofra J, Andia I, Padilla S, Mujika I. Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. Am J Sports Med 2007;35:245-51.

18 de Vos RJ, Weir A, van Schie HT, Bierma-Zeinstra SM, Verhaar JA, Weinans H, Tol JL. Platelet-rich plasma injection for chronic Achilles tendinopathy: a randomized controlled trial. JAMA 303:144-9.

19 Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. Am J Sports Med 2006;34:1774-8.

20 Peerbooms JC, Sluimer J, Bruijn DJ, Gosens T. Positive effect of an autologous platelet concentrate in lateral epicondylitis in a double-blind randomized controlled trial: platelet-rich plasma versus corticosteroid injection with a 1-year follow-up. Am J Sports Med ???? 38: 255-62.

21 Everts PA, Devilee RJ, Oosterbos CJ, Mahoney CB, Schattenkerk ME, Knape JT, van Zundert A. Autologous platelet gel and fibrin sealant enhance the efficacy of total knee arthroplasty: improved range of motion, decreased length of stay and a reduced incidence of arthrofibrosis. Knee Surg Sports Traumatol Arthrosc 2007;15:888-94.

22 Castricini R, Longo UG, De Benedetto M, Panfoli N, Pirani P, Zini R, Maffulli N, Denaro V. Platelet-rich plasma augmentation for arthroscopic rotator cuff repair: a randomized controlled trial. Am J Sports Med ?????; 39: 258-65.

23 Blair P, Flaumenhaft R. Platelet alpha-granules: basic biology and clinical correlates. Blood Rev 2009;23:177-89.

24 Longo UG, Ronga M, Maffulli N. Achilles tendinopathy. Sports Med Arthrosc 2009;17:112-26.

25 Egerbacher M, Arnoczky SP, Caballero O, Lavagnino M, Gardner KL. Loss of homeostatic tension induces apoptosis in tendon cells: an in vitro study. Clin Orthop Relat Res 2008;466:1562-8.

26 Yuan J, Murrell GA, Wei AQ, Wang MX. Apoptosis in rotator cuff tendonopathy. J Orthop Res 2002;20:1372-9.

27 Lian O, Scott A, Engebretsen L, Bahr R, Duronio V, Khan K. Excessive apoptosis in patellar tendinopathy in athletes. Am J Sports Med 2007;35:605-11.

28 Pearce CJ, Ismail M, Calder JD. Is apoptosis the cause of noninsertional achilles tendinopathy? Am J Sports Med 2009;37:2440-4.

29 Millar NL, Wei AQ, Molloy TJ, Bonar F, Murrell GA. Heat shock protein and apoptosis in supraspinatus tendinopathy. Clin Orthop Relat Res 2008;466:1569-76.

30 Scott A, Khan KM, Duronio V. IGF-I activates PKB and prevents anoxic apoptosis in Achilles tendon cells. J Orthop Res 2005;23:1219-25.

31 Longo UG, Franceschi F, Ruzzini L, Rabitti C, Morini S, Maffulli N, Forriol F, Denaro V. Light microscopic histology of supraspinatus tendon ruptures. Knee Surg Sports Traumatol Arthrosc 2007;15:1390-4.

32 Murrell GA. Using nitric oxide to treat tendinopathy. Br J Sports Med 2007;41:227-31.

33 Fredberg U, Stengaard-Pedersen K. Chronic tendinopathy tissue pathology, pain mechanisms, and etiology with a special focus on inflammation. Scand J Med Sci Sports 2008;18:3-15.

34 Fredberg U, Ostgaard R. Effect of ultrasound-guided, peritendinous injections of adalimumab and anakinra in chronic Achilles tendinopathy: a pilot study. Scand J Med Sci Sports 2009;19:338-44.

35 Toomey DP, Murphy JF, Conlon KC. COX-2, VEGF and tumour angiogenesis. Surgeon 2009;7:174-80.

36 Engebretsen L, Steffen K, Alsousou J, Anitua E, Bachl N, Devilee R, Everts P, Hamilton B, Huard J, Jenoure P, Kelberine F, Kon E, Maffulli N, Matheson G, Mei-Dan O, Menetrey J, Philippon M, Randelli P, Schamasch P, Schwellnus M, Vernec A, Verrall G. IOC consensus paper on the use of platelet-rich plasma in sports medicine. Br J Sports Med ????;44:1072-81.

37 Schepull T, Kvist J, Norrman H, Trinks M, Berlin G, Aspenberg P. Autologous platelets have no effect on the healing of human achilles tendon ruptures: a randomized single-blind study. Am J Sports Med ????;39:38-47.