The Role of Platelet Rich Plasma in Muscle Healing

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Conflict of interest: The authors declare no conflict of interest and do not have any financial disclosures.
ABSTRACT

The healing of a muscle injury is a complex and dynamic process characterized by different overlapping phases resulting in the restoration of the anatomic continuity and function. This process, triggered by the tissue injury itself, is modulated by different growth factors capable of directing the recruitment, duplication, activation and differentiation of different cell types. This key role played by different growth factors is the basis of the use of platelet-rich plasma in several circumstances, all of them characterized by the need of activating or ameliorating the process of tissue repair. There is extensive documentation of in vitro and in vivo studies demonstrating the safety and efficacy of growth factors in the muscle healing process. Unfortunately, for many different reasons, experimental results are usually difficult to interpret, clinical results are controversial and the relevance of use is still debatable. The present article aims to review the available scientific literature with particular focus on actual clinical applications.

Key words: Platelet-rich plasma, growth factors, muscle healing, recovery, muscle strain

Summary statement: The use of PRP is still a matter of debate. The review of the literature may guide physicians highlighting the actual clinical indications.
Introduction

Musculoskeletal injuries represent a challenging problem for Sports Medicine. Indeed, they account for the vast majority of all sport-related accidents and are the most common cause of severe long-term pain and physical disability (1). When considering muscle injuries, they are commonly classified on the basis of the mechanism of trauma. The direct forms are represented by laceration and contusion while the indirect forms are represented by muscle strains, which can be either complete or incomplete and are classically classified accordingly to their severity (2). Regardless of the damage mechanism, the healing process progresses through a series of overlapping phases resulting in the restoration of the anatomic continuity and function (3,4,5). This complex and dynamic process is characterized by a cascade of events, triggered by the tissue injury itself. Physiologically, healing progresses in a series of phases which include: the initial haemostasis, the acute inflammatory phase, the intermediate repair phase, and the advanced remodelling phase. The first stage starts with the formation of a blood clot and the consequent degranulation of platelets. The inflammatory phase, lasting up to 72 hours, is usually characterized by pain, swelling, redness, and increased local temperature. During this stage the aim of the treatment is to control bleeding as well as to minimise inflammation and pain. Non-steroidal anti-inflammatory drugs (NSAIDs) represent the common accepted treatment, and the only controversial aspect, when considering their use, is the appropriate timing of administration (6). Indeed, it has been suggested that it would be beneficial to delay NSAIDs treatment until 2–4 days after the injury since those molecules are known to be able to interfere with the chemotaxis of many cell types as well as with platelets aggregation, necessary mechanisms for the repair of the regenerating tissue (7). The repair phase lasts from 48 hours up to 6 weeks. During this period, muscle anatomic structures are restored with the involvement of several cell types. In particular, fibroblasts start to synthesise scar tissue while vessel neoformation occurs in order to bring nutrients to the healing area. This phase ends with the beginning of the wound contracture.

This brief description of the biology of the healing process highlights the fact that the complete recovery of a muscle strain usually occurs slowly and athletes are discouraged to resume their sport activity until walking without pain is possible (approximately 4 to 12 weeks). During this period, athletes usually follow different re-education programs. With this in mind, it has to be underlined that no consensus guidelines or agreed-upon criteria for a safe return to the previous level of sport activity are available (8,9). Moreover, it has been suggested that the long recovery period maybe also due to the structural alterations of the musculotendinous junction caused by the immobilization
after the accident (10). For this reason, it is commonly accepted that an early mobilization, followed by a rehabilitation programme, may facilitate an adequate structural resolution of the lesion (11). There is now a substantial amount of evidence accumulating to suggest that growth factors (GFs) may play a significant role during the muscle regeneration processes (12-15). Indeed, it has been clearly demonstrated that fibroblast growth factor (FGF), insulin-like growth factor-1 and 2 (IGF-1 and IGF-2), transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) are potent activators of myogenic precursor cells (16). It has also been demonstrated that some of these GFs are able to stimulate the differentiation and fusion of myotubes into multinucleated mature myofibers during the regeneration process (17-20). Conceivably, the demonstrated activity of many GFs during the healing processes is the basis of the concern expressed by several authors on the use of NSAIDs, which seem to negatively interfere with the repair process, in particular if administered during its early phase (12,13,16). Although the roles of all the previously listed GFs during the different healing stages are only partially known, the efficacy of many of these GFs has been extensively described. These observations represents the rationale of the use of platelet rich plasma (PRP) in several circumstances, all of which are characterized by the need to activate, modulate, speed up or ameliorate the process of muscle tissue repair (21).

**Platelets**
Platelets (PLTs) are small, non-nucleated cell fragments produced by large bone marrow precursors called megakaryocytes. PLTs circulate in the peripheral blood in a resting, inactive form for an average of 10 days. Normal PLT count range from 150.000/µL to 400.000/µL. The inactive PLT contains three types of internal granules: the dense granules, the lysosomes and the alpha granules. Each of these granules contains certain chemicals which are at the base of PLT function. To date, more than 300 different molecules have been identified in PLT granules (22). Dense granules predominantly contain small molecules (e.g., Calcium ions, serotonin, adenosine diphosphate, polyphosphates) mainly involved in the activation of other PLTs upon release. The lysosomes contain several enzymes that digest spent proteins and other metabolites. Alpha granules contain many different proteins representing the bulk of the PLT secretome. They include: hemostatic factors (e.g., Factor V, Von Willebrand Factor, fibrinogen), angiogenic factors (e.g., angiogenin, Vascular Endothelial Growth Factor), anti-angiogenic factors (e.g., angiostatin, Platelet Factor 4), growth factors (e.g., Platelet Derived Growth Factor, TGF-β, FGF, IGF-1, IGF-2, Epidermal Growth Factor), proteases (e.g., matrix metalloproteinase 2 and 9), necrotic factors (eg, TNF-α and β), as well as many other cytokines. Some of these molecules are produced by megakaryocytes and
packaged into granules. Other molecules are thought to be endocytosed by circulating PLTs themselves and then transported into α granules (23). This very large catalog of molecules released by activated PLTs suggests that the phase of PLT secretion could be pivotal in the establishment, as well as in the control and modulation, of the microenvironment at a wound site. However, to date it remains unclear whether this secretion phase is a controlled responsive process rather than a random, stochastic event (24). With this in mind, it is important to point out that skeletal muscle regeneration is characterized by the proliferation and differentiation of muscle precursor cells. The subsequent fusion with each other of these differentiated precursor cells leads to the formation of young multinucleated myotubes. Similarly to skeletal muscle development during embryogenesis, a precise control of proliferation and differentiation during regeneration is a critical phase for the generation of a functional tissue with the correct amount and types of cells (25).

**GFs effects during the muscle healing phases**

GFs are proteins acting through specific cell surface receptors on the appropriate target cell. The effect of each GF is usually related to its concentration as well as to the receptor sensitivity. On the base of their activity, GFs are classically divided into three groups, namely mitogen, chemo-attractant and transforming factors. Although the roles of all the GFs involved in the healing process remain only partially known, the efficacy of many of the GFs has been extensively demonstrated.

*Platelet Derived Growth Factor (PDGF)*

The binding of PDGF with its receptors determines the activation of the receptor tyrosine kinase, which leads to a cascade of biochemical events culminating in mitogenesis (26). It is well established that PDGF is a potent mitogen for fibroblasts and smooth muscle cells (27). Moreover it has been proposed that PDGF regulates myoblast proliferation and differentiation *in vitro*. It would seem therefore that PDGF has an important role in increasing the number of myoblasts during skeletal muscle regeneration (28).

*Fibroblast growth factor (FGF)*

FGF-1 and FGF-2 promote endothelial cell proliferation as well as the organization of endothelial cells into tube-like structures, beside the well known stimulation of the proliferation of fibroblasts. There is experimental data to show that FGF stimulates the proliferation and represses the terminal differentiation of satellite cells (29,30). In terms of muscle injuries, FGF-2 has been shown to be released from damaged muscle fibers and that FGF receptors plays a key role in the regulation of myogenesis (31). Moreover, it is known that FGF upregulates IGF-1 receptor expression in muscle
cells, thus suggesting a crucial role of FGF in the synergistic effect of different GFs during the healing process (32).

**Transforming Growth Factor-beta (TGF-β)**

TGF-β is a potent chemo-attractant for macrophages and stimulates or inhibits the growth of many cell types. The effects of TGF-β depend upon the interaction with other GFs (33). It has been shown experimentally that TGF-β stimulates the proliferation of undifferentiated mesenchymal cells, promotes the production of extracellular matrix, enhances the proliferation of fibroblasts, stimulates the biosynthesis of type I collagen, exerts synergic effects with other GFs, specifically PDGF, in the activation of satellite cells, stimulates endothelial chemotaxis and angiogenesis and inhibits macrophage and lymphocyte proliferation (34). When considering the process of muscle healing, it has been shown that TGF-β inhibits the proliferation and differentiation of myogenic satellite cell. Moreover, TGF-β is also involved in supporting the normal skeletal muscle architecture by regulating local collagen synthesis in tendon-related connective tissue (14,34-36).

**Vascular Endothelial Growth Factor (VEGF)**

There is experimental data to show that VEGF administration *in vitro* stimulates myoblast migration and survival, protects myogenic cells from apoptosis and promotes myogenic cell growth (37,38). In skeletal muscles, VEGF and its receptors are expressed in vascular structures but not in muscle fibres. Following experimental muscle injury, VEGF and its receptors were expressed in regenerating muscle fibres, suggesting the presence of an autocrine pathway promoting myocytes survival and regeneration. Moreover, VEGF administration with recombinant adeno-associated viral vectors significantly promoted muscle fibres regeneration in a dose-dependent manner (39).

**Insulin Like Growth Factor-1 (IGF-1)**

IGF-1 plays an important role in childhood growth and continues to exert anabolic effects in adults. IGF-1 has been shown to promote myogenic satellite cell proliferation and fusion (40). In addition, it has been demonstrated that local administration of IGF-1 to regenerating skeletal muscles enhanced muscle fiber enlargement during late regeneration (41).

**Epidermal Growth Factor (EGF)**

EGF and TGF-α have been shown to induce an equipotent stimulation of fibroblast migration and proliferation. Additionally, EGF provides an anti-apoptotic survival stimulus for satellite cells when they progress into a proliferative state (42). Some authors have shown that EGF promotes the
growth of the satellite cells and increases the proliferation of muscle-derived stem cells by increasing the number of mitotically active cells (43).

**PRP preparations**
The fact that PLTs alpha-granules contain several different GFs, present in physiological proportions, is an appealing feature when compared with the use of isolated GFs since it has been clearly demonstrated that many GFs act synergistically during the different phases of the healing process. Other advantages of the use of PLTs-derived GFs are represented by the fact that these preparations are relatively simple to obtain and handle with little or no risk of developing side effects. PRP is defined as a biological blood product obtained from the patient, which has anti-inflammatory and pro-regenerative functions (44,45) and is rich in GFs in physiologic proportions that act synergistically during the different phases of the healing process (12). A review of the scientific literature highlights the controversial nature of the clinical results involving PRP, thus making results difficult to interpret and more importantly, questioning the relevance of its use in clinical practice. While the debate intensifies, several limitations are particularly pertinent. Firstly, even if all PRP preparations contain a basic set of GFs, the relative concentration of each factor can differ among preparations. In addition, there was no proper terminology to classify and describe the many different variations of PLT concentrates (46). To overcome these limitations, the PAW classification based on the absolute number of PLT, the manner in which PLT activation occurs and the presence or absence of white cells was recently proposed (47). The debate continues with particular reference to the use of local anaesthetics and NSAIDs (48). We speculate that one should avoid the use of local anaesthetics in order not to modify the local pH, which is essential for the stability of several GFs and not to use NSAIDs in order not to reduce the first inflammatory response to injury, which is an essential step in the healing process.

**Efficacy of PRP preparations**
Numerous experimental and clinical studies have clearly demonstrated that myogenesis is not restricted to the prenatal period but also occurs during the regeneration of muscle tissue following injury (49). With this in mind, many authors demonstrated that *in vitro* PRP application to muscle cells resulted in an increased cell proliferation, satellite cells differentiation as well as in an increased synthesis of angiogenic factors (35,49,50). Studies carried out in animal models have demonstrated that PRP application enhanced muscle repair, inducing the proliferation of muscle cells, the differentiation of satellite cells and facilitating angiogenesis. Moreover, it has been clearly demonstrated that PRP application magnified the physiological early inflammatory response
following a muscle injury, modifying both the pattern of cellular recruitment and cytokine production. Finally, recent studies, clearly confirmed that PRP application produced a more pronounced increase of myogenic precursor cells together with an expansion of the myogenic cell pool necessary for myofiber formation and that positively modulated even the expression of stress response proteins, directly or indirectly correlating with the regeneration process (51-56). In a clinical context, it has been reported that the application of PRP into muscle injuries was able to reduce swelling and pain (57-59). Full recovery of functional capabilities was achieved in a smaller time when compared to other treatments and sonographic images showed fully regenerated muscle tissue following PRP treatment, in particular when considering sport related hamstring injuries (15,57,58,60-64). Initially, PRP administration was performed without any imaging guidance. More recently, it has been clearly demonstrated that the use of ultrasound-guided injections, preferably coupled with a peppering technique to distribute uniformly the preparation within the lesion, allowed a more precise visualization of the injury as well as the complete filling of the lesion itself with PRP. Moreover, the use of an ultrasound guide allows the optimal position of the needle as well as the adjustment of the procedure in real time (58,59).

The biological mechanisms explaining the improved muscle recovery following PRP treatment seem not to be limited to the actions of PRP on cellular growth and differentiation. The modulation of the inflammatory phase following the muscle injury is probably an important aspect of the PRP therapeutic action. As such, PRP has been shown to modulate secretion and recruitment of key inflammatory cells such as monocytes and leukocytes in the injury site. This hypothesis may explain, in part at least, the pain reduction in the first period following PRP administration as well as the early mobilisation of treated patients, which seems to be a crucial issue when considering the following rehabilitation approach as well as the time needed to fully return to the previous level of physical activity (52,65,66). Despite these encouraging results, some researchers have raised concerns that PRP treatment may increase the fibrotic healing response in muscle tissues, thus increasing the risk of re-injury. This idea is based on the observed elevation of TGF-β levels following PRP injection into muscle (67). Recent studies demonstrated that only extracellular signal-regulated kinases (ERK) activation was modulated by the presence of PRP while no effect on p38 mitogen-activated protein kinases (p38MAPK) and on protein kinase-b (AKT) activation was observed (53). Since it has been shown that constitutive activation of p38MAPK induced interstitial fibrosis (68), its early decrease following PRP administration strongly suggests a protective effect on regenerating skeletal muscle against fibrosis and therefore against the risk of re-injury.

Conclusions
There are numerous experimental and clinical studies that indicate a positive role of PRP during the healing process of muscle injuries. Nevertheless, a review of the literature reveals a lack of standardization when considering the preparation of PRP as well as its application. This observation may explain the difficulties in interpreting the clinical and experimental results obtained in different studies. At present, even if an “evidence based indication” cannot be derived from the literature, these studies do allow the following conclusions to be made:

1) The early treatment of a muscle injury with ultrasound guided injections of PRP is able to reduce pain and discomfort, particularly in the first weeks following the treatment, thus allowing an early mobilization of the patients. PRP application has to be considered as a valid therapeutic approach with the potentiality of significantly reducing the time and costs for making a complete functional recovery.

2) A coupled early mobilization is essential when considering the complete functional recovery.

3) An early treatment after the injury may results in better clinical responses.

4) It is suggested to avoid, when possible, the use of local anaesthetics in order not to modify the local pH, which is essential for the stability of several GFs and not to use NSAIDs in order not to reduce the first inflammatory response to injury, which is an essential step in the healing process.
References


