Platelet-Enriched Plasma and Muscle Strain Injuries: Challenges Imposed by the Burden of Proof

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Objective: To review the evidence for the clinical utilization of autologous plasma products in the management of muscle strain injuries.

Method: Systematic review using EMBASE and MEDLINE (up to March 2010).

Results: There is no level 1, 2, and 3 evidence for the use of autologous plasma products in muscle strain injuries. Furthermore, significant methodological limitations impact on the interpretation of the few published studies in this field.

Conclusions: Although basic science and the use of recombinant growth factors in animal models support the concept of applying growth factors to acute muscle injuries, it is unclear if this evidence can be directly translated to reflect outcomes from platelet-enriched plasma. There remain a large number of unanswered questions, including the principle questions regarding safety and efficacy, which require appropriate scientific investigation. It is incumbent on sports physicians wishing to enhance athlete care, together with researchers, to search for these answers.

Key Words: muscle injury, platelet-rich plasma, autologous plasma, platelet-enriched plasma

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INTRODUCTION

Muscle strain injuries continue to result in a high morbidity within professional and amateur sport. Despite the high prevalence of muscle strains, there is a limited evidence base for the majority of management techniques, and the treatment, in particular minimizing the risk for recurrent muscle injuries, has progressed little in the past 30 years. Moreover, although numerous risk factors for muscle injury have been identified,[1,2] evidence suggests that the greatest risk factor for a recurrence remains a previous injury to that muscle, perhaps a result of scar tissue formation at or near the injury site.[3] The exact etiology for recurring injuries remains to be determined, and the optimization of both preventative and management techniques for an initial muscle injury remains a high priority.[4]

Increasingly, invasive injection techniques have been proposed in the management of muscle strain injuries with the theoretical goal of minimizing inflammation and fibrosis, while maximizing myofiber regeneration. This approach has created a considerable controversy within the specialty of sports medicine.[5,6] The use of autologous plasma products, containing elevated concentrations of platelets, growth factors (GFs), and other substances, is one such technique.[7] Based on a limited number of animal model studies that have shown a positive impact of isolated recombinant GF on muscle regeneration,[8,9,10] the application of autologous platelet concentrates to an injured muscle is thought to accelerate regeneration, thereby enhancing healing and minimizing reinjury.[11] The use of autologous plasma as a source of GF seems attractive because it is easily obtainable with simple apparatus and is relatively affordable.[12] Moreover, its use has rapidly gained the support of the popular media as a result of its purported "natural" properties, high level of efficacy, and lack of side effects.[13,14] From January 2011, the World Anti-Doping Agency have removed intra-muscular autologous platelet concentrates from their prohibited list, thereby potentially increasing its availability in elite sport. However, despite its elevated public profile and theoretical benefits, there remain many unanswered questions surrounding the use of these techniques in the management of muscle injuries, and the burden of proof remains with scientists and practitioners to confirm or refute the clinical utility of this technology.[6,15]

Unfortunately, despite its increasing popularity as a treatment for soft tissue injuries, there remains neither a uniform terminology nor an understanding as to what constitutes platelet-enriched plasma (PEP).[11] Terminology in common usage includes platelet-rich plasma (PRP) and plasma (preparation) rich in GFs (PRGFs); however, many of these terms are associated with commercial products and will be avoided where possible in this discussion. This systematic review will provide a cogent summary of the status of autologous plasma products as they relate to muscle injury and repair. Accordingly, fibrin gel products not appropriate for the simple application to acute muscle injuries, and which may have a distinct bioavailability to liquid PEP, are not discussed herein.[16,17,18]

METHODOLOGY

To assess the current state of the evidence for autologous plasma injections and treatment of muscle strain injuries, we

performed a systematic review of the literature using EMBASE and MEDLINE databases (up to and including March 2010) via the University of Queensland Library. All titles were assessed by the senior author (B.H.H.) as having any reference to autologous plasma injections and skeletal muscle injury or healing and were included as relevant if this was the case. Once determined to meet the criteria, both authors were to systematically review the articles and assess their methodological rigor by the Judet criteria. Reviews were subsequently excluded. As can be seen from the search strategy and outcomes (Table 1), this systematic review failed to uncover any relevant titles. Our initial focus was on clinical trials, but given the lack of literature in this area, this was extended to include all sources, using relevant references cited in review articles but not retrieved using the above searches. This technique uncovered 3 relevant human references to the use of PRP or autologous GFs. Given the apparent lack of high-level evidence base, a review of the basic science surrounding the use of autologous plasma injections in muscle is provided, with conclusions drawn.

CLINICAL EVIDENCE FOR THE USE OF PLATELET-RICH PLASMA IN MUSCLE STRAIN INJURIES

Human Studies

The first human clinical description of the use of this technology in skeletal muscle tissue was not actually using plasma but rather autologous conditioned serum (ACS).[19] A sample of 50 ml of whole blood was withdrawn from patients, conditioned in a specially designed tube to increase the GF content, and stored at $-20°$ until being used. Eighteen professional athletes suffering from muscle strains to the hamstring, adductor, iliopsoas, gluteus, abdominal oblique, gastrocnemius, and rectus femoris muscles were nonrandomly recruited into the study and compared with 11 previously treated professional athletes (control) with anatomically similar injuries. All injuries were classified as moderate (grade II) and treatment in both groups started within 3 days of the injury. Local anesthetic (LA) and 5 mL of ACS were injected, with subjects undergoing a mean of 5.4 treatments. The control subjects were managed with injections of Actovegin (Nycomed, Vienna, Austria) and Traumeel (Heel GmbH, Baden-Baden, Germany), with the mean number of treatments being 8.3. Five mL of intramuscular LA was injected with each treatment. Both study

and control subjects underwent rehabilitation and were prescribed oral homeopathic medication. Primary outcome was the ability to return to competitive sport, as determined by nonblinded physical therapists and physicians. Although this study reported a significant reduction in return-to-play time (16 vs 22 days), the large number of limitations of the study restrict its interpretation: nonblinded, atypical control, use of LA, variable injury site with no quantification of injury grade, no long-term follow-up, and no measurement of GF levels in the actual injectate. Collectively, these factors unfortunately suggest that this study provides little more than a glimpse at what the outcomes of using this technology may provide.

Available as a conference abstract only, Sanchez et al[20] presented the results of 20 professional athletes with muscle injuries injected with an autologous PRGF, compared with 25 age-matched and sex-matched controls. Injury severity was assessed using ultrasonography, and any hematoma was evacuated before PRGF injection into the injured area. The number of injections was determined by the size of the injury (small tears, 1 injection, and medium to large tears, 2 to 3 injections), and all underwent physiotherapy. No further details on the methodology were available. The authors concluded that PRGFs reduced pain and swelling, with functional recovery in "half of the expected recovery time." Ultrasonography revealed evidence for enhanced regeneration and no fibrosis, and no reinjuries occurred after return to play. Unfortunately, again a lack of details concerning methodology, outcomes, and follow-up limit the interpretation of this article.

A single case report was retrieved regarding the use of PEP in muscle injuries. Loo et al[21] presented a case of a 35-year-old male professional bodybuilder with a clinically and ultrasound-confirmed right adductor longus strain injury. Using autologous plasma activated with calcium, an unknown amount of PRGF was infiltrated weekly into the injury site for 3 weeks. One week after the final injection, the athlete was able to return to competitive training. Unfortunately, a lack of detail regarding the grading of the injury, timing of the injection, associated treatment, follow-up, and training demands imposes significant limitations to this report.

Animal Studies

Before their trial on sportsmen, Wright-Carpenter et al[22] conducted a study using 108 mice and ACS injections. Conditioned serum [containing an elevation in fibroblast growth factor (FGF)-2 and transforming growth factor-β1 (TGF-β1) compared with nonconditioned] and the same

TABLE 1. Search Methodology and Systematic Search Results

Search Number	Search Strategy	No. Citations	No. Relevant Citations
1	platelet′/exp/mj AND rich AND ′plasma′/exp/mj AND [humans]/lim	72	0
2	preparation AND rich AND in ′growth′/exp/mj AND factors AND [humans]/lim	15	0
3	platelet rich plasma′/exp/mj AND [humans]/lim	396	0
4	platelet′/exp/mj AND rich AND ′fibrin′/exp/mj AND [humans]/lim	28	0
5	platelet′/exp/mj AND concentrate AND [humans]/lim	236	0
6	#1 OR #2 OR #3 OR #4 OR #5	707	0
7	muscle′/exp/mj AND ′injury′/exp/mj AND [humans]/lim	6213	Not assessed
8	#6 AND #7	2	0

volume of saline (control) were injected into the gastrocnemius muscle at 2, 24, and 48 hours after injury. Results suggested that satellite cell activation was increased by 84% at 30 and 48 hours after injury in the ACS-treated animals. Furthermore, by day 7, there was an unquantified increase in centrally nucleated myofibers and a quantifiable increase in the proportion of large-diameter fibers compared with the control, both measures of myofiber regeneration. By day 14 after injury, this difference between the treated and control animals had resolved. This study provides some support for the use of ACS to assist in the histological regeneration of muscle after a contusion injury. It is unclear, however, whether this had any effect on muscle function. Moreover, whether these results would translate to a strain injury is not known from this study.

In 4 skeletally mature sheep, Carda et al[23] surgically lacerated spinal muscles and immediately either filled the wound with an autologus preparation activated with calcium chloride, purportedly rich in GFs (PRGFs), or closed the wound without support. Histological comparison of the wound site between the 2 groups was made at 4 to 6 days and 3 to 5 weeks. Limited abstract interpretation suggests a finding of enhanced muscle regeneration in the PRGF-treated animals. No analysis of the injectate was performed to confirm the contents.

Hammond et al[24] used 72 rats to study the effect of PRP in 2 types of muscle injury. Using a muscle strain injury induced in the tibialis anterior (TA) by superimposing a maximal isometric contraction onto either a single lengthening contraction (large strain) or a series of lengthening contractions (small strain), the effect of PRP on large or small strains was assessed, respectively. In injured rats, 100 μL of PRP was injected into the TA, and platelet-poor plasma (plasma with reduced concentrations of platelets) was used as the control, on days 0, 3, 5, and 7 after injury. Maximal isometric torque was measured before each injection and then on days 14 and 21, with the multiple lengthening injury model (smaller strain) paradoxically resulting in a longer healing time. The PRP injection resulted in a significant functional improvement in the single lengthening injury model at day 3 and in the multiple lengthening protocol at days 7 and 14. Of note, myoD and myogenin messenger RNA transcripts, markers for satellite cell activation, were elevated after injury but significantly more in the PRP-treated injury. Furthermore, centrally nucleated fibers were more frequent in the PRP-treated injuries in the multiple repetition injury, reflecting elevated myogenesis.[25] Of interest, the single repetition injury model did not result in any increase in central nucleated fibers (evidence for muscle regeneration), with or without PRP. Hence, at least in an animal model of a minor muscle strain, this article provides good evidence for an improvement in muscle regeneration with PRP. However, the lack of benefit in a large single muscle injury, which results in sarcolemma disruption and increased tissue degeneration,[25] perhaps more frequent in sports people, remains a conundrum.

PLATELET FUNCTION AND PLATELET-ENRICHED PLASMA

To understand the implications of using PEP on injured tissue, it is critical to have a clear understanding of platelet function. Derived from the megakaryocyte under the stimulation of numerous factors, platelets are anuclear cells with a life span of 7 to 10 days[26] but which retain other organelles containing numerous substances critical in hemostasis, inflammation, and tissue repair.[27,28] Recognized intraplatelet organelles include mitochondria, a dense tubular system (DTS), dense granules, α-granules, and lysosomes. As a result of their anuclear state, platelets do not synthesize the contents of granules, rather they retain them from their megakaryocyte stage, but may also either passively or actively take up intragranular substances from their surrounds.[28,29,30] The DTS contains calcium and other enzymes critical to the activation of platelets, whereas the dense granules contain proaggregatory, proinflammatory factors such as nucleotides (eg, adenosine triphosphate and adenosine diphosphate), bioactive amines (serotonin and histamine), and calcium. However, unlike the DTS, dense granule calcium is not involved in the initial activation process.[27,28] Lysosomes, which incompletely release their contents on activation,[31] contain proteases, glycosidases, and other degrading enzymes that are predominantly active in an acidic environment,[28] typical in the early phases after muscle injury.[32] Alpha-granules are, however, the most abundant organelle with approximately 50 to 80 α-granules per platelet,[29] and these contain a range of factors, including GFs. Platelet-derived growth factor, TGF-β, vascular endothelial growth factor, hepatocyte growth factor, and epidermal growth factor are known to be present in the α-granules; however, the exact GF content of these granules is yet to be determined. When activated, dense granules, α-granules, and lysosomes discharge their contents into the surrounding medium[27,28,31,33] via a cytoskeleton-dependent mechanism.[34]

PLATELET ACTIVATION

In vivo, platelet activation is preceded by adhesion, which can result from exposure to collagen, von Willebrand factor, fibrinogen, and other factors.[35] Subsequent activation may be precipitated by factors such as thrombin or thrombin receptor agonists,[33] calcium, collagen,[36,37] specific metalloproteinases,[38] complement, catecholamines, serotonin,[35,39] or shear stress.[40] This activation is dependent on specific platelet membrane glycoproteins binding to ligands, kinase activation,[38] and cytoplasmic calcium influx from both the DTS[28,36] and the extracellular milieu.[34] This intracellular calcium influx results in alterations in the microtubular arrangements[34] within the platelet and subsequent translocation of granules to the membrane surface. Hence, exposed collagen from a myofascial tear or intramuscular tendon exposure could theoretically result in aggregation and activation of platelets contained within PEP infiltrated into the lesion; however, in vivo evidence for this is lacking. Interestingly, however, platelets are not homogeneous in their morphology, with a range of platelet densities observed, corresponding to variable α-granule volume.[41] P-selectin, a glycoprotein expressed on the surface of platelets on activation and reflecting α-granule fusion with the cell membrane,[28] has been shown to have higher levels in low-density platelets.[41] As a result, low-density platelets may

potentially release greater amounts of platelet granule content, including GF, creating a GF expression gradient within any pool of platelets. Furthermore, recent evidence suggests that platelet α-granules themselves are heterogenous in nature, containing distinct subpopulations of GF, released in response to specific signaling.[42] Subsequently, proangiogenic and antiangiogenic factors may be stored and released in distinct subsets of α-granules, in response to distinct signaling pathways.[30] This heterogeneity of α-granules has significant implications on both the interpretation of GF levels between studies using differing activation methods and the potential impact of the local environment on activation and hence timing of application of PEP, platelet activation, and selective GF release.[32] For example, pre-infiltration activation of PEP with calcium or thrombin may result in unregulated release of α-granule contents, with both proregenerative and antiregenerative factors being released. There is marked variability in the use of activated or nonactivated PEP in the literature, which may impact on both the GF expression and the clinical outcome.[43,44,45,46]

AUTOLOGOUS PLATELET-ENRICHED PLASMA

Autologous PEP is formed from the separation of whole blood into its plasma and red cell constituents, with the subsequent concentration of platelets into a small volume of plasma. Separation is frequently achieved with varying degrees of centrifugation but may equally occur via cell separator apparatus.[47] Typically, the platelet concentration in whole blood is in the range of 150 to 400×10^6 per milliliter,[26] but in autologous concentrated plasma, platelet levels may increase up to 8-fold.[43] It remains unclear what level of platelet concentration is either representative of or optimal in PEP, and although levels of 600 000 to 1 000 000 platelets per microliter are frequently touted, there remains limited evidence for this approach.[46,48,49,50,51] With an increasing range of products available for production of PEP, it is unclear whether levels such as this are actually required, particularly when one considers the low correlation between platelet levels and observed GF concentrations.[43,52] The levels of various GFs measured in different PEP preparations varies markedly, with limited correlation to platelet count and seems rather to be dependent on the combination of both physiological considerations and plasma preparation methodology.[18,43,53,54,55,56,57] When this variability is combined with the lack of clinical trials in which either platelet or GF levels have actually been measured (let alone other active platelet factors), it is difficult to form any consensus on optimal PEP formulations, particularly for use in muscle injuries.

In addition to the variability in platelet contents observed in PEP, the presence of white cells in the plasma will also depend on the separation methodology used.[18,55,58,59,60] Moreover, it remains unknown as to whether the presence of white cells in PEP should be considered of benefit to or an impediment to healing.[47] Advocates of the former argue that there is an anti-infective benefit of white cells being present,[27,61,62] whereas the latter argue that the proinflammatory nature of white cells will be counterproductive to healing.[50] Although this is consistent with the current understanding of the potential negative effects of inflammatory mediators on muscle healing,[63] it is of interest to note that similar proinflammatory mediators as are found in white cells and are also released from both the lysosomes and α-granules of platelets on activation.[28,29,31] Hence, it remains unclear what impact PEP, with or without white cells present, will have on the inflammatory cascade after muscle injury.[24,64]

Growth factors have numerous roles in tissue repair and regeneration, and it is these factors that are widely considered to account for the purported beneficial effects of PEP. However, as illustrated above, although there is good, albeit limited, evidence for the role of isolated recombinant GFs in muscle regeneration,[8,9] little is known of the impact on muscle regeneration of either the bolus release of GFs from activated platelets,[64] or other products released from the α-granules, dense granules, and lysosomes on platelet activation. Significantly, however, it is recognized that the uncontrolled leakage of GFs from platelet α-granules, as observed in gray platelet syndrome, results in excessive fibrosis and deposition of collagen in the bone marrow.[65] This knowledge illustrates the importance of temporally controlled release of α-granule contents, in an appropriate environment.

CLINICAL CONSIDERATIONS WITH THE USE OF PLATELET-ENRICHED PLASMA IN MUSCLE

At a most superficial level of consideration, the use of PEP seems to be a valid tool in the treatment of muscle injury. As outlined above, however, the clinical and scientific support for its use is lacking. Furthermore, PEP should not be thought of as a benign "physiological" substance,[11,49] but rather a manipulated and supraphysiological (albeit autologous) product with properties potentially quite distinct from its original state. It remains speculative at best when determining which components of PEP actively play a role in muscle healing, particularly when one considers the oversimplification being applied to most discussions of its use. Furthermore, although we continue to uncover more details of specific GFs and their functions (eg, FGF alone is now known to have at

TABLE 2. Some Unanswered Questions Regarding the Use of PEP in Muscle Strain Injuries

Does PEP enhance muscle regeneration?

Does PEP reduce recovery time from muscle strain injury?

What are the indications for PEP utilization?

Which are the active GFs in a PEP solution?

How do the GFs interact with each other in an acute or chronic injury?

Is timing of application important?

What concentrations/volumes of PEP are required?

How many applications of PEP are optimal?

Does the platelet concentration really matter?

Does the system utilized matter?

Do you need to activate the PEP before application?

Should you aim to exclude all white cells?

Is whole blood just as effective?

What is the role of exercise and rehabilitation after PEP infiltration?

What are the short-term and long-term side effects of PEP?

Is there a supraphysiological performance enhancing effect of PEP infiltration in muscle?

least 22 variations[66]), our understanding of the complex nature of GF functions and interactions in PEP preparations remains in its infancy. Hence, when considered in detail, there remain a large number of unanswered academic and clinical questions with regard to the use of PRP preparations in muscle strain injuries (Table 2).

The physiological impact on an acute muscle injury of a bolus infiltration of an unknown concentration of platelets, GF, and other factors as is found in any PEP preparation is scientifically unknown. Specifically, animal studies have recently suggested that by comparison with sustained release of GF, a bolus dose of recombinant GF is not as effective for muscle healing.[64,67] Unlike platelet matrices, which are felt to act in a sustained release manner,[16] up to 90% of GFs may be released from PEP in the first hour after activation.[49,68] Taken together, this may suggest that a bolus of PEP will be ineffective. Furthermore, although it may seem intuitive that the administration of a "physiological" range of GFs will be better than a single GF application for tissue regeneration,[12] this concept has been challenged in studies using combinations of GFs in tendons.[69] It remains unclear if the timing (as observed in tendon healing[70]) or dose[49,69] of the PEP infiltration will be critical to muscle regeneration. For example, 2 to 3 weeks after an injury, the environmental milieu may preferentially upregulate platelet TGF-β activity, thereby favoring fibrosis over regeneration,[71] and may therefore be at least theoretically contraindicated at that time. Each muscle has distinct anatomical and physiological characteristics, and in rabbit ligaments, the medial collateral ligament and anterior cruciate ligament have distinct GF response profiles to injury.[72] This may account for the variability observed in injury recovery from different muscle injuries,[73] but as a result, it remains unknown if all muscles and grades of muscle injury heal with the same GF requirement.[24] Furthermore, although the physiological milieu and collagen exposure in acutely injured muscle tissue should be sufficient to activate platelets, it is unknown if this is the case or whether preinfiltration activation is preferential. Finally, what is the role of rehabilitation, and how should this be affected by the infiltration of PEP? Evidence from rat Achilles tendon studies suggests that without appropriate active rehabilitation, the benefit of infiltration with GFs is negated,[74] and one may expect the same outcome in muscle.

Regarding the safety of PEP, little is known. Bovine thrombin used in early trials of PEP has been recognized to cause an immune response resulting in life-threatening coagulopathies,[75] and so is no longer used. Autologous thrombin or other activating agents, such as calcium chloride, have eliminated this risk. Theoretical risks, such as neoplastic change, increased fibrosis, and infection, have not been quantified and require quality studies with long-term follow-up.

CONCLUSIONS

Despite a high public profile, the use of autologous plasma injections in the management of acute muscle strain injuries has no clinical evidence base. Although basic science and the use of recombinant GFs in animal models support the concept, it is unclear if this evidence can be directly transposed

to reflect outcomes from PEP. There remain a large number of unanswered questions, including the principle questions regarding safety and efficacy, which require appropriate scientific investigation. It is incumbent on sports physicians wishing to enhance athlete care to search for these answers.

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