JOURNAL OF PHYSICAL EDUCATION RESEARCH

Journal of Physical Education Research, Volume 4, Issue I, March 2017, pp.68-77
ISSN: Print-2394 4048, Online-2394 4056, Impact Factor: 0.519, IBI Factor: 4.29, GIF: 0.765

PLATELET ENRICHED PLASMA FOR ACUTE HAMSTRING MUSCLE STRAINS

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How to cite this article: Abdelgaber, A.M. (March, 2017). Platelet enriched plasma for acute hamstring muscle strains. Journal of Physical Education Research, Volume 4, Issue I, 68-77.

Received: July 12, 2016 **Accepted:** March 23, 2017

ABSTRACT

Hamstring muscle strains are among the most common injuries in sport, but despite increasing research into the epidemiology, aetiology and management the rates of both injury and re-injury remain high. Typically, hamstring injury management is conservative, but recently the use of autologous platelet enriched plasma (PEP), has been proposed as a treatment tool which may optimise muscle regeneration and enhance clinical outcomes. Unfortunately, there remains little scientific evidence for the clinical use of these techniques in muscle injuries. This report outlines the current clinical evidence for the use of PEP in muscle injuries. A case report of a patient with a grade II semi-membranosus muscle strain, injected with PEP while concurrently using platelet inhibitors will illustrate the clinical, radiological and theoretical challenges of this new technique. Further clinical research into the clinical utility of PEP in muscle injury is required and it is incumbent on sports physicians and researchers to address this research deficit, if PEP is to live up to its high public profile.
Keywords: *Muscle, autologous plasma, platelet enriched plasma, platelet rich plasma.*

1. INTRODUCTION

Hamstring muscle strains are among the most common injuries in sport, with a resultant high morbidity (Verrall, Slavotinek, Banes, Fon, & Esterman, 2006). Appropriately, a great deal of time and energy has been spent researching both the epidemiology of hamstring injuries and the implementation of preventative programmes (Brockett, Morgan, & Proske, 2004; Askling, Saartok, & Thorstensson, 2006; Gabbe, Branson, & Bennell, 2006; Arnason, Andersen, Holme, Engebretsen, & Bahr, 2008). Despite this, both the rates of injury and re-injury remain high (Hagglund, Walden, & Ekstrand, 2006). Typically, hamstring

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injury management is conservative (Drezner, 2003), based around progressive restoration of range of motion, strength and functional activities (Arnason *et al*., 2008: Drezner, 2003; Gabbe *et al*., 2006), although there remains a paucity of literature to support the majority of conservative management programmes (Sherry & Best, 2004). Recently, a variety injection techniques including the use of autologous platelet enriched plasma (PEP), have been touted to enhance the regenerative properties of the muscle following a strain injury (Wright-Carpenter, Klein, Schaferhoff, Appell, Mir, & Wehling, 2004; Creaney & Hamilton, 2007; Orchard, Best, Mueller-Wohlfart, Hunter, Hamilton, Webborn, …., & Glasgow, 2008; Hammond, Hinton, Curl, Muriel, & Lovering, 2009; Loo, Lee, & Soon, 2009). At this stage however, there remains little scientific evidence for the clinical use of these techniques.

First developed in the mid 1990's in maxillofacial surgery, the technique of deriving plasma concentrated in both platelets and growth factors (GF) is now widely used in some fields of medicine and is considered to have many potential applications (Anitua, Sanchez, Orive, & Andia, 2007; Marx, 2004). However, the use of autologous plasma remains a relatively novel approach for enhancing muscle healing (Creaney & Hamilton, 2007; Foster, Puskas, Mandelbaum, Gerhardt, & Rodeo, 2009). The application of specific recombinant GF, such as Insulin-like Growth Factor-1 (IGF-1), basic Fibroblast Growth Factor (bFGF), Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor-AB (PDGF-AB), has been shown to enhance muscle healing, with increased myofibre regeneration, augmented angiogenesis and ultimately increased strength (Efihimiadou, Nikolettos, Lambropoulou, Papadopoulos, & Kontoleon, 2006). However, the timing and the sequence of activation of specific GF during the healing process is highly sensitive and critical to optimisation of muscle regeneration (Menetrey, Kasemkijwattana, Day, Bosch, Vogt, Fu, Moreland, & Huard, 2000). This concept challenges the appropriateness of bolus doses of autologous PEP injections, containing variable concentrations and proportions of GF (Ehrenfest, Rasmusson, & Albrektsson, 2009), in the management of such a carefully controlled sequence of events, as occurs in muscle healing. Furthermore, in an animal model bolus doses of GF have been shown to have little impact on muscle healing (Borselli, Storrie, Benesch-Lee, Shvartsman, Cezar, Lichtman, Vandenburgh, & Mooney, 2009). Notwithstanding this complexity, concentrates of platelets and GF may be simply prepared from the centrifugation of whole blood, thereby markedly reducing their cost and increasing their clinical availability (Anitua, Sánchez, Nurden, Nurden, Orive, & Andía, 2006). Although the routine use of GF injections now appears a financially viable therapeutic option, technical issues (Ehrenfest *et al.*, 2009) may continue to limit its use, and to date it has been used by a limited number of practitioners in Sports Medicine.

Furthermore, the World Anti-Doping Agency (WADA) has prohibited the use of intra-muscular autologous plasma injections, presumably due to safety and efficacy concerns (WADA, 2009).

While GF are produced from a variety of tissues, a large number of the GF found in the plasma are released from alpha granules, following the activation of platelets (Nurden, 2007). Activation of platelets may be achieved in vivo via contact with collagen (Roberts, McNicol, & Bose, 2004) or in vitro utilizing thrombin and/or calcium (Landesberg, Roy, & Glickman, 2000; Roussy, Duchesne, & Gagnon, 2007). Somewhat paradoxically however, it has been shown that platelet inhibitors such as Clopidogrel and Aspirin do not affect the levels of GF obtained from PEP (Smith, Binford, Holt, & Webb, 2007). However, there is no evidence to evaluate either the clinical risks or benefits of PEP use in soft tissue injury in those patients utilizing platelet inhibitors. In this illustrative case we sought to determine the clinical and radiological outcomes of undergoing PEP therapy for a hamstring muscle strain injury, while using platelet inhibitors.

2. METHODS AND MATERIALS

2.1 Case History

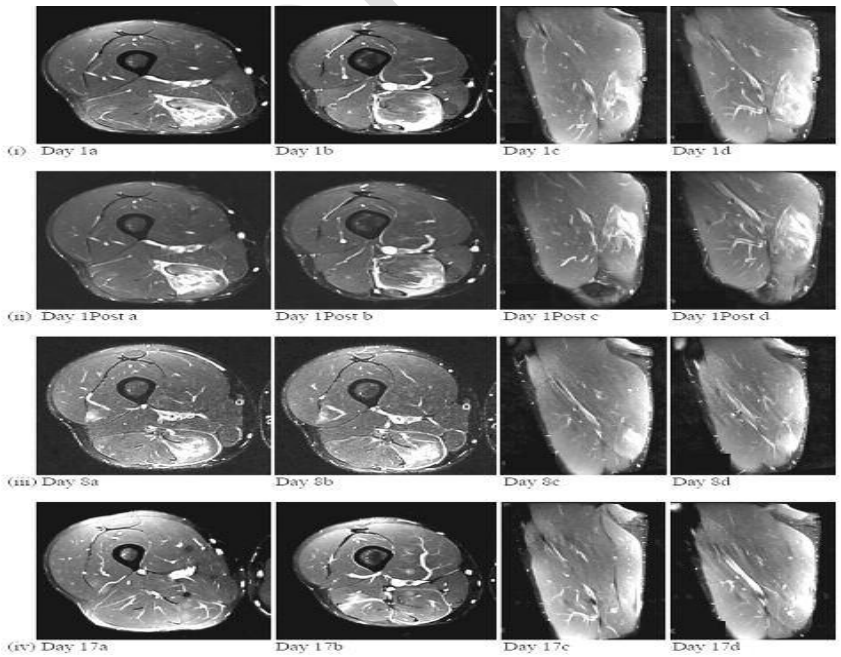
A 42-year-old physically active male (DH) presented 24 hours after experiencing the sudden onset of right sided posterior thigh pain following an unanticipated eccentric contraction while surfing behind a power boat. He had a long history of recurrent low grade bilateral hamstring muscle strains, grade III right adductor longus strain, bilateral calf strains, and a ruptured Achilles tendon. All injuries except the ruptured Achilles tendon were treated conservatively, with complete resolution of symptoms. At the time of injury he was four months post stenting of his right anterior descending coronary artery, following an inferior myocardial infarction sustained while mountain bike racing. Consequently, he was taking Clopidogrel (Plavix® 75 mg/day), Aspirin (100 mg/day) and Atorvastatin Calcium (Lipitor® 40 mg/day).

On examination, DH was found to have an antalgic gait, pain with mild isometric contraction and a straight leg raise of 30° limited by pain. He was tender to palpation over the middle and distal thirds of the medial hamstring, and a clinical diagnosis of a grade II hamstring strain injury was made. An MRI scan (fig 1 (i)) confirmed marked intra and extra muscular signal enhancement (oedema and haemorrhage) consistent with a grade II semimembranosus muscle strain injury.

Following discussion of the diagnosis and treatment options, and after providing written consent, DH elected to undergo an injection of autologous

plasma into the hamstring. This was undertaken as part of a pilot for a larger study approved by the institutional ethical committee. The subject had 27 ml of blood withdrawn from an antecubital vein and the plasma separated into PEP and platelet poor (PPP) fractions, with a commercially available centrifuge based separation system (Biomet Recover™, USA). Using a sterile ultrasound (US) guided technique, 3 ml of PEP was infiltrated using a 23 gauge needle into the region of maximal tenderness and muscle injury (as determined by the MRI, ultra-sound and palpation), using 3 separate depots of 1 ml. The region was then iced for 15 minutes and was immediately followed by a repeat MRI to evaluate the impact of the injection therapy (fig 1 (ii)). The results of this evaluation indicated that there was no substantial change from the pre-injection MRI. The patient was discharged and advised to ice for 20 minutes every 1-2 hours, and between icing to keep it compressed for 48 hours.

Figure 1: Sequential T2 weighted axial (a and b) and sagital (c and d) MRI scans of the right hamstring on (i) Day One, (ii) Day One (Post Injection), (iii) Day Nine and (iv) Day Seventeen post injury respectively, illustrating the rapid resolution of signal hyperintensity. Note the additional oedema in Biceps Femoris (Day 17b) reflecting Delayed Onset Muscle Soreness resulting from the volume of strengthening work being performed



The day following the injection, DH began a comprehensive daily physiotherapy programme. This initially consisted of simple range of motion exercises, regular

ice therapy and rapidly progressed through a programme of isometric, concentric and eccentric exercises, as pain free motion allowed. In addition, core stability training was included in the overall rehabilitation programme. He had no residual effects from the injection at any point in the rehabilitation.

Nine days following the initial injury (eight days post injection), he underwent a repeat MRI (fig 1 (iii)), which suggested only mild resolution of both intra and extra-muscular oedema. Clinically DH was progressing well, with a full range of pain free motion, other than residual pain on isometric con- traction at the extremes of range, and ongoing focal tenderness.

Seventeen days following the injection, DH had a full range of motion, was pain free in maximal isometric contraction and had no residual tender- ness. A repeat MRI at day 17 (fig 1 (iv)) revealed complete resolution of the signal enhancement. DH continued to make good progress with regular strengthening of the injured area, and after 3 weeks was performing all his recreational activities (i.e., cycling, swimming, running) pain free.

2.2 Autologous Plasma Injectate Analysis

Approximately three weeks following the initial injury, further blood was withdrawn from DH, to assess the platelet concentration and growth factor levels. Twenty seven ml of whole blood was fractionated into PEP and PPP samples (Biomet Recover™) and the platelet numbers were deter- mined in each fraction (Coulter Count; Cell-Dyne 3700, Abbott, IL, USA). This method achieved a five-fold enrichment of the platelets (Platelet Count: Whole blood 253 × 109/l; PPP 27.9 × 109/l; PEP 1278 × 109/l White Cell Count: Whole Blood 5.4 × 109/l; PPP 0.016 × 109/l; PEP 27.8 × 109/l).

The PEP samples were separated into two aliquots; one being immediately frozen at -80oC for subsequent GF analysis, and the other undergoing activation with CaCl2 (25mM) for 1h at 37o C. Following activation the samples were centrifuged 4000 rpm, 4o C for 10 min and the fibrin clot was separated from the supernatant. The supernatant was aliquoted and frozen at -80° C until analysis. GF release, at baseline and following activation, was evaluated in PEP samples by commercial ELISAs (R&D Systems, Oxon, UK). All inter- and intra- assays coefficients of variation were < 10%.

3. RESULTS

Results of the platelet and growth factor analysis are shown in table 1.

Table 1: GF levels in basal and activated PEP compared to published reference ranges

	Activated PEP (pg/ml)	Reference Ranges (pg/ml)
PDGF AB	77,664.8	39,000-602,000 [30, 32-44]
IGF-1	148,800	4,500-200,000 [32, 33, 36-38, 45-48]
HGF	1,582	
VEGF	1,780	0-4,000 [29, 32, 33, 44, 46, 48, 49]

4. DISCUSSION

Despite intensive pre-habilitation and rehabilitation programmes, hamstring muscle strains continue to be one of the most common injuries in all sports with a high morbidity. Optimal management of the hamstring muscle injury continues to be debated, and invasive injection techniques remain controversial (Cook, 2009; Franklyn-Milller, Etherington, & McCrory, 2010; Orchard *et al.*, 2008). The use of homeopathic products such as Traumeel™, biomedical products such as Activegan™ (Orchard *et al.*, 2008) and autologous concentrated plasma have been suggested to enhance soft tissue healing, although scientific support is limited (Creaney & Hamilton, 2007; Foster *et al.*, 2009).

This case report illustrates a safe and efficacious application of platelet concentrated plasma in the management of a grade II semimembranosus strain. However, while the clinical recovery was satisfying, it remains within the normal range of recovery times for the activities in which DH was involved; albeit in the authors' experience at the rapid end of the recovery spectrum. Unfortunately, a single case report allows no clear indication as to the benefits of the PEP injection to be gleaned from the pure clinical outcome, other than that the out- come was highly satisfactory, and lacking in negative outcomes (follow-up to 12 months).

Notwithstanding the clinical outcome, the rapid resolution of both extra and intra muscular oedema and haemorrhage from the injured area by 17 days appears remarkable. In our experience, and that of the literature, it is common for residual oedema to remain within the muscle even after an injured athlete has returned to full functional activity (Connell, Schneider-Kolsky, Hoving, Malara, Buchbinder, Koulouris, Burke, & Bass, 2004; Koulouris & Connell, 2006). MRI has been shown to be more sensitive than US for detecting ongoing muscle activity, and altered MRI signal intensity may remain positive for at least six weeks following an injury (Connell *et al.*, 2004). Hence, we were surprised in this case, that the injury had MRI resolution at 17 days, and further evaluation is warranted as to whether this was a consequence of the infiltration with PEP. This observation has been previously made using autologous conditioned serum, and presents a useful outcome tool for prospective studies of muscle healing (Wright-Carpenter *et al.*, 2004). It remains unclear whether residual oedema in a muscle is an actual risk for recurrence or a contra-indication to return to play (Connell *et al.*,

2004; Orchard, Best, & Verrall, 2005), and for this reason, most practitioners will rely on progressive clinical and functional assessment in their serial evaluations, rather than imaging findings (Wright-Carpenter *et al.,* 2004). It is clear however, that the absence of oedema on MRI is of reassurance to both the patient and practitioner.

While numerous factors are released from the platelet a-granules, lysosomes and dense granules upon activation, it is the GF that are presumed to have the active effect. While the role of individual recombinant GF in muscle regeneration is gradually being clarified (Menetrey *et al.,* 2000), it remains unclear which GF in the milieu of PEP will up-regulate muscle regeneration at any given time point. Furthermore, as illustrated in Table One, there is a great variability in GF levels reported from PEP, increasing the scientific uncertainty as to what are the key elements of PEP. Despite this patient being on platelet activation inhibitors, we have shown that his platelets released increased concentrations of GF upon expo- sure to CaCl, in the range expected for PEP. This is consistent with a previous report (Smith *et al.*, 2007), but for the first time we have illustrated a successful clinical outcome of a patient concurrently using platelet inhibitors and who is treated with PEP for a muscle injury.

The complete resolution of the increased signal intensity, the satisfactory clinical progression and the lack of complications support the use of PEP in this type of injury. Despite this many questions remain regarding the use of PEP in muscle strain injuries, including its method of action, optimal dosing, optimal timing of application, long term efficacy, relevance of platelet and GF concentrations, the need for pre- application activation and safety profiles.

Perhaps one of the most intriguing questions as we start to explore the clinical efficacy of this technique is how can one rationalize the use of a single bolus of a broad spectrum complex of unknown GF in the management of a process which normally involves the carefully timed and selectively activated application of the body's own GF. It is considered that injected PEP will act in a bolus dose manner with the majority of platelets degranulating and GF being released immediately (Marx, 2004). Recently, using a rat model of muscle injury, it has been illustrated that while slow release growth factors may be beneficial to muscle regeneration, bolus doses of autologous growth factors showed no such benefit (Borselli *et al.*, 2009). Furthermore, how does an injured tissue select the appropriate GF for the time point in its healing process, while switching off the less temporally appropriate GF? Despite the large number of unanswered questions, the early observations of this technology in the management of muscle strain injuries encourage and challenge us to explore this further.

The use of complex GF preparations remains a novel but exciting technique in the management of soft tissue injuries. The World Anti-Doping

Agency have concerns regarding the abuse of such technology in elite athletes, resulting in its inclusion on their prohibited list (WADA, 2009), and further research in this area is required, before it can be recommended for use in the general public or elite athlete arena.

5. CONCLUSIONS

The use of autologous plasma and GF technology in the management of soft tissue injury has some support from in vitro and animal studies. While its clinical use in muscle injuries remains in its infancy, it has the potential be a technically and fiscally viable tool for the practitioner. However, the technique requires further comprehensive evaluation of its indications, efficacy and safety before any conclusions can be drawn.

6. ACKNOWLEDGMENTS

The authors would like to acknowledge the tireless support of Sirine Boukarroum in the collection and collation of data.

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