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Single dose of intra-muscular platelet rich plasma reverses the increase in plasma iron levels in exercise-induced muscle damage: A pilot study

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Abstract

Objectives: Platelets rich plasma (PRP) therapy is widely used in enhancing the recovery of skeletal muscle from injury. However, the impact of intramuscular delivery of PRP on hematologic and biochemical responses has not been fully elucidated in exercise-induced muscle damage (EIMD).

Design: Moderately active male volunteers participated in this study and were assigned to a control group (CONTROL, n=6) and platelet rich plasma administration group (PRP, n=6).The subjects performed exercise with a load of 80 % one repetition maximum (1RM) maximal voluntary contraction of the elbow flexors until point of exhaustion of the non-dominant arm was reached. The arms were treated with saline or autologous PRP post-24h EIMD. Venous blood samples were obtained in the morning to establish a base-line value and 1-4 days post-exercise and were analyzed for serum ferritin, iron, iron binding capacity (IBC), creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT).

Results: The baseline levels of plasma iron, ferritin, IBC, CK, LDH, AST and ALT were similar in both the control and PRP groups. However, 24 h following exercise a significant increase in these parameters was observed in both groups between 1-4 days during the recovery period. Interestingly, PRP administration decreased plasma iron levels compared to the control on the second day post-exercise. Plasma IBC increased in PRP group from day 2 to 4 post exercise compared to the control group whilst PRP administration had no effect on plasma ferritin, CK, AST, ALT and LDH.

Conclusions: Acute exhaustive exercise increased muscle damage markers, including plasma iron, IBC and ferritin levels, indicating muscle damage induced by exercise. PRP administration

improves inflammation by reversing the increase in the iron levels post-exercise without displaying any myotoxicity and may have a role to play in the recovery of exercise-induced muscle damage.

Key Words: platelet-rich plasma, plasma iron, ferritin, exercise-induced muscle damage

1. Introduction

Recently platelet-rich plasma (PRP), an autologous derivative of whole blood containing a supraphysiological concentration of platelets has gained increasing popularity in both the scientific literature and the wider media for its potential application in the treatment of traumatic musculoskeletal and sports-related injuries, cancer biology, and dermatology. In addition, it has been reported that PRP administration may improve recovery from tendon and muscle injuries[1,2]. Biologic healing utilizes the normal mechanisms for tissue repair and incorporates these at the site of injury. Blood components such as platelets migrate to the injury site and play an important role in tissue repair. Platelets contain various growth factors and cytokines that initiate and promote healing by stimulating cell migration, cell proliferation, angiogenesis, and matrix. Other important bioactive factors released from platelets include histamine and serotonin and these platelet growth factors enhance DNA synthesis, chemotaxis, angiogenesis, increase collagen deposition, and stimulate synthesis of extracellular matrix[3].

It is well established that an unaccustomed and strenuous exercise in the trained and untrained individual can induce skeletal muscle damage[4]; this phenomenon is commonly known as “exercise-induced muscle damage” (EIMD) and is determined by the type, intensity and duration of exercise[5]. Moreover, in sports, the eccentric/concentric type of exercise has been used as a specific training model for muscle strength improvement during training sessions. However, symptoms of EIMD include reduced muscular force, increased stiffness, swelling delayed onset muscle soreness (DOMS), and an increased blood activity of muscle proteins such as creatine kinase (CK> 10.000 IU/L)[4], alanine transaminase (ALT)[6], aspartate transaminase (AST)[6], lactate dehydrogenase

activity[7] and this may have a negative impact on performance[4]. Moreover, EIMD initiates an inflammatory response associated with secondary muscle damage and remodeling[8] since during the acute phase, both neutrophils and phagocytic macrophages can release reactive oxygen and nitrogen species and remove debris by phagocytosis[9]. Moreover, recent studies have reported the levels of the iron-regulatory hormone hepcidin are also increased after exercise[10-12]. Hepcidin is a liver-produced peptide hormone, up-regulated in response to elevated iron levels and the inflammatory cytokine interleukin-6 (IL-6)[13,14] and an increase in hepcidin levels usually occurs as a homeostatic response to inflammatory stimuli namely the IL-6 or elevated iron levels[13]. Peeling et al. reported that inflammation, hemolysis, serum iron, ferritin, and urinary hepcidin were elevated in the high intensity interval post- running session[11]. As such, the post-exercise hepcidin response is likely to be homeostatic in nature, to help control and reduce the elevated levels of serum iron resulting from the exercise-induced hemolysis[15].

Many studies have been published proposing various methods for treating DOMS, including cryotherapy, anti-inflammatory medication, stretching, hyperbaric oxygen, homeopathy, ultrasound, L-carnitine, rest, light exercise and electromagnetic shields[16-21]. For example, non-steroidal anti-inflammatory drugs (NSAIDs) are routinely prescribed to alleviate EIMD-related symptoms and restore normal physical function of the muscle[22]. However, it has been reported that NSAIDs act by blocking COX and thus they may have a detrimental effect on muscle regeneration and super-compensation[20]. Moreover, there is strong to moderate evidence that intramuscularly injected local anaesthetics and NSAIDs are myotoxic. The administration of PRP has also been reported to induce myotoxicity, however the evidence is conflicting and further studies are required to confirm this as well as the possible myotoxic effects of corticosteroids[23]. Furthermore, clinical and histopathological studies have shown the potential myotoxicity of intramuscular injections in both animals and humans[24,25], resulting in pain at the injection site and histopathological changes of

inflammation, necrosis and fibrosis[24,25]. Besides histological changes, the local plasma creatine kinase (CK) concentration is the most commonly used valid marker for skeletal muscle myotoxicity[26-28]. There is conflicting evidence regarding the myotoxicity of intramuscular PRP injections. Two studies used an animal muscle injury model and reported increased signs of regeneration, less necrosis and less granulomatous tissue in the muscles injected with, PRP[29,30] and autologous conditioned serum (ACS)[31]than in control muscles on histological evaluation for up to 2 weeks. However, information regarding the myotoxicity of intramuscular PRP injection or the cross-talk between hematologic and biochemical response has not been reported in exercise-induced muscle damage. Therefore, we hypothesized that intramuscular PRP injection might improve inflammation and beneficial effect on DOMS and muscle damage induced by exercise without mytoxicity effects. The objective of the present study was to investigate whether the myotoxicity effects of the intramuscular PRP injection can provide an effective recovery strategy for attenuating DOMS and muscle damage induced by high-intensity muscle exercise in humans.

2. Methods

2.1. Study Design

Twelve moderately active male volunteers participated in this randomized double-blind placebo-controlled trial to verify the effects of the intramuscular PRP injection on hematologic, biochemical response and mytoxicity on muscle recovery after an eccentric/concentric exercise. Subjects were randomly placed into two groups: PRP (n=6) and CONTROL (n=6) and they had not been involved in any regular weight-training program and had no history of injury to the arm, shoulder and elbow region. The nature and the risks of the experimental procedures were explained to the subjects, and signed informed consent to participate in the study was obtained. Before the test session, participants were examined and checked by the use of routine blood analysis by a

medically qualified practioner. Ethical approval was obtained from The Balikesir University Medical Faculty Ethics Committee (2013/14) and each participant gave written informed consent prior to the study.

2.2. Muscle Damage Exercise Protocol

For the exercise-induced muscle damage test, subjects were seated on a bench with their arm positioned in front of their body and resting on a padded support, such that their shoulder was secured at a flexion angle of 0.79 rad (45°) and their forearm was maintained in the supinated position throughout the exercise. Subjects were repeatedly weight-loaded upon dumbbell lowering to achieve a 80% of maximum voluntary contraction (MVC), 2-min rest between the sets of elbow extension from the flexed position at 90^0 to fully extended position slowly over 5 s, until exhaustion was experienced. The subjects were also given verbal encouragement by the investigator to maintain constant speed throughout the procedure. They were instructed to continue their normal activities and to abstain from any strenuous exercise at least two weeks before the experiment. Moreover, they were asked to continue their usual food intake, not to change the amount or frequency of dietary meat and not to use any dietary supplements, anti-inflammatory drugs, or anything else that could affect muscle soreness and damage until the end of the study.

2.3. Platelet-rich plasma and placebo

Each participant based on computerized randomization lists to either receive placebo (saline) injection or PRP injection in non-dominant arms with post-24h DOMS exercise. PRP preparation was obtained from eight millilitres of peripheral blood which was drawn from the dominant arm and the samples were centrifuged for 9 minutes at 3500 revolutions per minute (H-19F, RegenCentrigel) according to manufacturers recommendation (Regen ACR-C, Regen Lab, Switzerland). Subsequently, 4 ml of PRP was injected using a 20-gauge needle into the pain full region of the non-dominant arm under sterile aseptic conditions. This kit produces 4 mL of PRP

from 8 mL citrated blood. Therefore final platelet concentration is approximately ≤2 fold over whole blood platelet concentration. Platelet recovery is reported to be > 95% and a leukocyte recovery of 58% .

Venous blood samples were collected pre-, and 4 days post-exercise, and analyzed for complete blood counts (WBC, RBC, Hb) , serum ferritin, iron (Fe), iron binding capacity (IBC), creatinine kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) as markers of muscle damage and inflammation.

2.4. Hematological analysis

For analyses of serum iron (Fe), iron binding capacity (IBC),and ferritin blood samples were collected without any additive and after centrifugation sera were stored at-20°C until analyzed. Iron and IBC were measured spectrophotometrically in an Advia 1800 analyzer (Siemens Healthcare, Erlangen, Germany) and ferritin was measured by immunoturbidimetric assay in an Olympus AU400 analyzer (Beckman Coulter, CA, USA).

2.5. Biochemical analysis

Following centrifugation at 825 *xg* for 10 min, serum was analyzed for ALT, AST, CK and LDH activities using commercially available kits in a chemistry autoanalyser (Cobas Integra 800; Roche Diagnostic GmbH; Mannheim, Germany).

2.6. Statistical Analysis

All calculations were performed using SPSS software (SPSSInc, Chicago, Illinois, USA). The values of serum Fe, IBC, ferritin, ALT, AST, LDH, and CK are presented as raw values as the area under the curve (AUC) during the experimental period. The AUC was calculated as the sum of four or five trapezoid areas separated by each supplement time point. Two-way mixed model analyses of variance (2 group X 5 times) with repeated measures were used. Differences in continuous variables between groups were assessed using Independent t-test and between multiple

172 points within the same group were analyzed using student's paired t-test. Data are expressed as
173 means ± SE and the level of significance was set at $p<0.05$.
174
175 **3. Results**
176 There was no difference in body weight, height, age and exercise performance had no
177 significant differences between PRP and CONTROL (Table 1, $p >0.05$). The baseline values for
178 plasma Fe, IBC, ferritin, AST, ALT, LDH and CK values were similar between the CONTROL and
179 the PRP administered group ($p >0.05$). However, 24 h following exercise, the plasma Fe, IBC,
180 AST, ALT, LDH and CK values significantly increased in the CONTROL and the PRP
181 administered group on day 1 to 4 post exercise muscle damage ($P< 0.01$, Fig. 1-5). Acute
182 exhaustive muscle exercise also increased the plasma ferritin levels in control and PRP group ($P<$
183 0.001 and $P< 0.05$) respectively, Fig.3. Interestingly, PRP administration decreased plasma iron
184 levels ($P= 0.002$) compared to the control group but this was only observed on the day 2-3 post-
185 exercise-induced muscle damage (Fig. 1). Moreover, plasma IBC levels were increased in PRP
186 group from day 1 to 4 post exercise compared to control group ($P < 0.05$, Fig. 2). In contrast, PRP
187 administration had no effect on plasma ferritin (Fig. 3), AST, ALT (Fig. 4), LDH and CK levels
188 (Fig. 5).
189 **Insert Table 1 here**
190 **Insert Figure 1 here**
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4. Discussion

In this paper we report the effect of single dose of intra-muscular platelet rich plasma on iron levels in in exercise-induced muscle damage. This was a pilot study in which 6 subjects participated in the control group and test group. Acute exhaustive exercise increased muscle damage markers, including plasma iron, IBC and ferritin levels were increased confirming exercise-induced muscle damage. PRP administration resulted in improved muscle recovery from injury without displaying any myotoxicity. Many methods have been utilised for the treatment of DOMS, including cryotherapy, anti-inflammatory medication, stretching, hyperbaric oxygen, homeopathy, ultrasound, L-carnitine, rest, light exercise and electromagnetic shields[16-19]. Inflammatory conditions have been essentially treated by the use of non-steroidal anti-inflammatory drugs (NSAIDs) although they are ineffective in reducing muscle pain and do not increase muscle performance during DOMS[20-22,32-34]. As an alternative to conventional treatments, platelet-rich therapy has been applied due to its potential in accelerating muscle healing and reducing a player's injury time. As far as we are aware, this study is the first to examine the effect of intramuscular PRP administration on DOMS and muscle damage markers also post exercise-induced muscle damage during the recovery period in healthy human volunteers. Importantly, our results show that the acute exhaustive muscle exercise increased the plasma iron, IBC and ferritin level in both groups. Our findings on plasma iron, IBC and ferritin response to acute exhaustive exercise are in agreement with previous reports[10-13]. which also reported an increase in serum iron, IBC and ferritin levels which was linked to the intensity of the exercise. It seems the increase in serum ferritin levels also led to an increase in plasma iron and hepcidin levels. A majority of publications have reported an elevated hepcidin levels 24 h post exercise, preceded by acute increase in serum iron and inflammation parameters[10-13, 35]. However, we did not measure the post- exercise hepcidin levels in this study. The present study indicates that post-exercise serum iron, IBC and ferritin levels are

induced as a result of inflammatory response due to exhaustive muscle exercise. Speculatively, acute exhaustive exercise increased the free iron that enters the plasma and has a reduction-oxidation (redox) potential, which may promote free radical formation as a result of Fenton and Harber–Weiss reactions and can result in oxidative damage to tissues[36]. Interestingly, in this study it was observed that PRP administration reversed the observed increase in plasma iron level due to muscle damage 2-3 days post-exercise. Furthermore, plasma IBC levels were up-regulated in PRP group from day 1 to 4 post-exercise. On the other hand PRP administration depressed the plasma ferritin levels during the recovery phase compared to control values, but did not reach statistical significance. These results are novel and to the best of our knowledge, no data exists concerning the acute effect of intramuscular PRP administration on plasma iron, IBC and ferritin levels parameters during recovery period in an acute exercise-induced muscle damage model. In general, related studies have reported that PRP treatment has anti-inflammatory properties through its effects on the canonical nuclear factor κB signalling pathway in multiple cell types including synoviocytes, macrophages and chondrocytes[37]. In addition, PRP treatment has suppressed tendon cell inflammation *in vitro* and *in vivo*, marked by the upregulation of COX-1, COX-2 and mPGES-1 expression with highly PGE2 production[38]. Additionally, the present study demonstrated that intramuscular PRP injection plays a key role as an anti-inflammatory by suppressing effect of increased free iron in plasma during the muscle damage recovery. Evidently, we have previously shown that elbow flexors muscle strength peak torque values were improved after PRP administration when compared to the control arm, this occurred on the same day (second day) when the serum iron level declined post exercise-induced muscle damage *(Unpublished data).*

Serum CK concentration is the most sensitive indicator of muscle damage and it begins to rise approximately 2-12 h after the exhaustive exercise. Exhaustive physical exercise increases serum enzyme activities such as CK, AST, LDH and ALT hence these are considered as markers

for the muscular damage derived from intense exercise[39]. The increased activities of CK and LDH in serum after exhausted exercise could act as signals, attracting neutrophils to the damaged muscle and initiating the inflammatory response. The maintenance of high CK activities after recovery could be and indicator of muscle repair[40-42]. Our result demonstrate that CK, AST, ALT and LDH levels increased post-exercise during the DOMS period in both groups, indicating muscle damage. On the other hand, CK concentration is the most commonly used valid marker for skeletal muscle myotoxicity in the intramuscular injections[26-28]. Although intramuscular PRP injections are commonly used, there is only limited evidence base for myotoxicity in animals models[29-31] and these studies reported increased signs of regeneration of the muscle whilst necrosis and granulomatous tissue was decreased in the muscle injected with PRP when compared to the control, however, no response to CK levels was reported in these studies[29-31]. Limited human studies have been shown that intramuscular injection of lidocaine[43] and bupivacaine (20 ml)[44] lead to an increase in CK levels. Our findings showed that the plasma level of CK was increased in response to exhaustive exercise, however, PRP administration did not alter CK levels in the PRP group compared to control. Hence, intramuscular PRP injection did not show mytoxicity in exercise- induced model.

5. Conclusion

Our study results indicate that an acute exhaustive exercise increased muscle damage markers, including plasma iron, IBC and ferritin levels, indicate muscle damage due to exercise-induced. PRP administration improved the inflammatory resoponse by reversing the observed increase in iron levels and may have a role to play in the recovery of exercise-induced muscle damage. Evidently, intramuscular PRP injection had no effect on CK levels, indicating that it is not myotoxic.

Conflict of interest

There are no conflicts of interest including financial, personal or other relationships with other organizations.

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