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RevaTen platelet-rich plasma improves cardiac function after myocardial injury☆

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Abstract

Objective: Cell therapy is an exciting area of investigation for repair of injured myocardial tissue. Platelet-rich plasma (PRP) is an autologous fractionation of whole blood containing high concentrations of growth factors including vascular endothelial growth factor and insulin-like growth factor, among many others. PRP has been shown to safely and effectively enhance healing of musculoskeletal tissue primarily by reparative cell signaling. Despite a growing body of evidence on PRP's safety and efficacy, limited studies have been performed using PRP in cardiovascular tissues. Utilizing a murine myocardial permanent ligation and ischemia/reperfusion model, this study sought to determine whether RevaTen PRP (Menlo Park, CA, USA), a proprietary formulation of PRP, improves cardiac function as measured by left ventricular ejection fraction (LVEF).

Methods: Via thoracotomy, the left anterior descending arteries (LAD) of 28 mice were occluded by suture either permanently or for 45 min to induce ischemic injury and then reperfused. Mice undergoing permanent ligation had intramyocardial injections of either RevaTen PRP (n=5) or phosphate-buffered saline (PBS; n=4). Magnetic resonance (MR) imaging was performed to calculate LVEF at 7 days. Mice undergoing ischemia and reperfusion had intramyocardial injections of either PRP (n=10) or PBS (n=9) and underwent MR imaging to calculate LVEF at 21 days. Hearts were harvested for histologic examination following imaging.

Results: Compared with PBS controls, RevaTen PRP-treated animals that underwent LAD ligation had a 38% higher LVEF 7 days after injury (PRP=36.1±6.1%; PBS=26.4±3.6%, P=.027). Compared with PBS controls, PRP-treated animals who underwent ischemia–reperfusion of the LAD had a 28% higher LVEF 21 days after injury (PRP=37.6±4.8%, control=29.3±9.7%, P=.038). Histologic analysis suggested the presence of more scar tissue in the control group compared to the PRP-treated animals.

Conclusion: MR imaging demonstrated a positive effect of RevaTen PRP on left ventricular function in both a ligation and ischemia–reperfusion murine model. Our results suggest RevaTen

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PRP should be investigated further as a potential point-of-care biologic treatment following myocardial injury.

Keywords: Platelet-rich plasma; Myocardial infarction; Congestive heart failure; RevaTen; Platelet cell therapy, Ischemia, Reperfusion; Heart attack

1. Introduction

Despite advances in prevention, pharmacological intervention, and medical procedures, myocardial infarction remains the leading cause of death in industrialized nations [1]. Thus, investigators continue to search for potential biologic therapies to treat damaged myocardial tissue. Although stem cell therapies currently represent an exciting area of research, challenges include cell choice, mode of harvesting and processing, and cell survival. Platelet-rich plasma (PRP) is a form of platelet cell therapy that has emerged as an interesting biologic tool in regenerative medicine. Its benefits include point-of-care availability, simple autologous preparation, and no rejection risks. PRP is a fractionation of autologous whole blood that contains significant quantities of a variety of growth factors including, but not limited to, vascular endothelial growth factor, transforming growth factor-beta (TGF-β), insulin-like growth factor, and platelet-derived growth factor [2]. PRP can be considered a form of autologous cytokine therapy.

PRP has been evaluated as a primary treatment or in conjunction with surgery in a variety of tissues. PRP has been shown to improve the biomechanical properties of tendons, enhance their vascularity, and induce migration of reparative cells to a site of injection [3–6]. There is also significant evidence supporting the use of PRP in chronic, severe lateral epicondylitis [7–9]. Other investigations have studied its use in the treatment of rotator cuff [10], Achilles tendon [11–13], and patellar tendon [14] injuries. However, only limited work has been performed evaluating the potential benefits of PRP on cardiovascular tissues. Preclinical studies have been limited to improvement of perfusion in a murine hind limb ischemia model [15] and modulation of post-myocardial infarction remodeling in a rodent model [16]. One clinical study using PRP in patients with chronic angina demonstrated its excellent safety profile [17]. Although PRP has been evaluated and used in a variety of conditions, the most clinically relevant cardiovascular applications have yet to be determined.

Notably, not all PRP is the same. Some forms of PRP contain only concentrated platelets. Others also contain increased white blood cells compared to baseline whole blood. Ex vivo activation of PRP with thrombin and/or calcium produces a gel that can be applied to a surgical site. In vivo activation of PRP via direct contact with collagen-containing tissue is a preferred form of PRP because it results in slower growth factor release at the desired anatomic site [7,8]. In light of this, Mishra et al. (in press) have proposed a classification system that describes different formulations of PRP and provides a framework for comparing how these different types of PRP function. RevaTen PRP is a proprietary formulation of unactivated PRP containing both highly concentrated platelets and white blood cells. In this investigation, we specifically sought to evaluate how in vivo application of RevaTen PRP influences left ventricular cardiac function after myocardial injury as measured by magnetic resonance imaging using a murine model.

2. Methods and materials

2.1. Animal model

Six- to 8-week-old female NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice (Jackson Laboratory, Bar Harbor, ME, USA) were housed at the Stanford University animal care facility under standard temperature, humidity, and timed-lighting conditions, and provided mouse chow and water ad libitum. All animals were handled in compliance with the National Research Council's guidelines for the care and use of laboratory animals. A total of 28 mice were selected for two experiments. In both models, human PRP was delivered via intramyocardial injection into severe combined immunodeficient (SCID) mice. With the use of well-established ischemic/reperfusion injury and myocardial infarction models, SCID mice were selected to preclude an immunologic response to the human PRP. SCID mice have impaired VDJ recombination and, consequently, an inability to make T cells, B cells, or components of the complement system; however, they have a functional innate immune system (natural killer cells, macrophages, neutrophils). This murine strain is routinely used in vivo to study the behavior of human cells (humanised mice), where human genes are expressed by mouse cells or transferred human tissue. This approach has been exploited in cancer biology, autoimmunity, allergy, infections, and transplantation research [18]. In the first experiment, nine mice were randomly assigned to experimental (PRP-treated, n=5) or control (PBS-treated, n=4) groups in a permanent ligation model. In the second experiment, 19 mice were randomly assigned to experimental (PRP-treated, n=10) or control (PBS-treated, n=9) groups in an ischemia–reperfusion model.

2.2. Human platelet-rich plasma

RevaTen platelet-rich plasma was prepared from whole human blood from the same donor using a proprietary separation device and process (BioParadox, Menlo Park,

CA, USA). After preparation, PRP was buffered to physiologic pH using 8.4% sodium bicarbonate and delivered to the myocardium without exogenous activation. The platelet and white blood cell counts were calculated for each trial before and after the PRP was prepared.

2.3. In vivo myocardial permanent ligation model

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice were anesthetized and maintained with 3% isoflurane, intubated, and placed on a rodent ventilator. Permanent left anterior descending artery ligation was performed via a left lateral thoracotomy. Fifty microliters of human PRP (experimental group, n=5) or 50 μl of PBS (controls, n=4) was divided into two directly visualized intramyocardial injections in the ischemic region via a 27-gauge needle. The injections were delivered 5 min after artery ligation. The animals were sacrificed on Postoperative Day (POD) 7.

2.4. In vivo myocardial ischemia–reperfusion model

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice were anesthetized using the same protocol described in the ligation model. Ischemia and reperfusion of the left anterior descending artery were performed via a left lateral thoracotomy and an 8-0 Ethilon suture. The presence of ischemic myocardium confirmed adequate occlusion. After an occlusion time of 45 min, reperfusion of the LAD was allowed for 15 min. Fifty microliters of human PRP (experimental group, n=10) or 50 μl of PBS (controls, n=9) was subsequently divided into two intramyocardial injections in the ischemic region via a 27-gauge needle. The injections were delivered 5 min after artery ligation. Animals were sacrificed on POD 21.

2.5. Myocardial function assessment

Mice were anesthetized with 2% isoflurane with 1 l/min oxygen and placed in the supine position. A small animal ECG and respiratory gating system (Small Animal Instruments, Stony Brook, NY, USA) were applied to acquire images at the end of each QRS and end respiration. Magnetic resonance images were performed on a Signa 3.0 T Excite HD scanner (GE Health Systems, Milwaukee, WI, USA) with a customized small animal surface coil.

Gated gradient-echo fast spoiled GRASS (FSPGR) sequences were used to acquire sequential short-axis slices spaced 1 mm apart from apex to base of the mouse heart. For each sequence, 20 cine frames encompassing one cardiac cycle were obtained with the following sequence parameters: echo time=4.6 ms, number of excitations=2, field of view=50×50 mm, matrix=256×256, flip angle=60°.

A contouring program, Fujin Plus 08 version 3 (Tokyo, Japan), was used to trace the endocardial border of the LV myocardium for each slice of the heart over the entire cardiac cycle in order to determine ejection fraction (EF). This technique has previously been used to evaluate LV function after injection of embryonic stem cells to treat acute myocardial infarction [19]. Following imaging, animals were euthanized and hearts harvested and processed for histology (including hematoxylin and eosin) and trichrome staining.

3. Results

3.1. Platelet-rich plasma

PRP and baseline whole blood were analyzed for platelet and white blood cell counts. RevaTen PRP contained an average platelet concentration 5.06 times baseline (P=.025) and an average white blood cell concentration 3.6 times baseline (P=.019).

3.2. LVEF increases after intramyocardial PRP as determined by MRI

To determine the effect of PRP on LV function following myocardial infarction, mice underwent permanent ligation of the left anterior descending artery and were treated with either intramyocardial PRP (n=5) or PBS (n=4). PRP-treated animals had a significant improvement in left ventricular function compared to control [left ventricular ejection fraction (LVEF) 36.1±6.1% vs. 26.4±3.6%, PRP vs. control] at 7 days (P=.027). This increase represented a 38% relative improvement in cardiac function in favor of the PRP-treated group (Fig. 1).

To determine the effect of PRP following ischemia–reperfusion injury, mice underwent temporary left anterior descending artery occlusion and then were subsequently treated with either PRP (n=10) or PBS (n=9). Similar to the myocardial infarction model, LV function significantly improved in PRP-treated animals at POD 21 (LVEF 37.6±4.8% vs. 29.3±9.7%, PRP vs. control, P=.038). This difference represented a 28% relative improvement in EF in favor of the PRP-treated group (Fig. 2).

The software analysis tool for cardiac magnetic resonance images did not allow for direct calculation of ventricular volumes but only a ratio of volumes. LVEF was obtained from the ratio of these volumes. There was qualitative

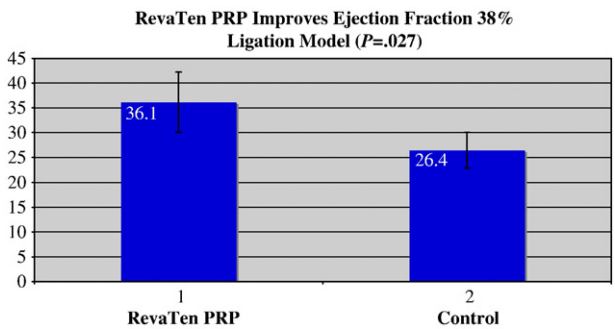

Fig. 1. Ligation model data.

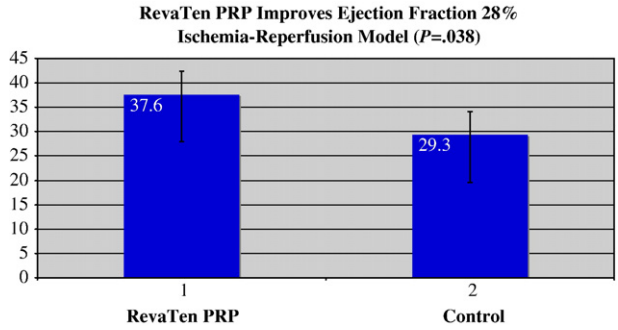

Fig. 2. Ischemia-Reperfusion model data.

evidence of less dilatation and a lack of negative ventricular remodeling in the RevaTen PRP-treated hearts (Fig. 3).

3.3. Histologic analysis

Hearts were harvested on POD 21 and processed to examine myocardial fibrosis. Histologic analysis suggested the presence of more scar tissue in the control group compared to the PRP-treated animals as detected with trichrome staining (Fig. 4).

4. Discussion

The use of biologic therapies in conjunction with standard reperfusion protocols to preserve or repair injured myocardium has recently become a major focus of cardiovascular investigation. Among these novel biotreatments, adult stem cell therapies such as the transplantation of mononuclear bone marrow cells, mesenchymal bone marrow cells, and skeletal myoblasts have shown promise based on studies demonstrating improved EF and/or decreased infarct size [20–23]. However, difficulty with cell availability, cell harvest morbidity, and timing of delivery has limited the adoption of these approaches. The cost and potential risks of these approaches are also high and potentially prohibitive.

Our results suggest that RevaTen PRP, a formulation containing concentrated platelets and white blood cells, improves cardiac function as measured by LVEF after myocardial injury. In the permanent ligation model, a 38% improvement in LVEF was demonstrated using RevaTen PRP at 7 days compared to controls. Similarly, in an ischemia–reperfusion model, a 28% improvement in LVEF was shown using RevaTen PRP at 21 days compared to controls. These represent significant positive functional changes in two clinically relevant models. The results meet or exceed cell therapy in terms of efficacy when compared to two of the largest clinical studies of mononuclear bone marrow cell therapy without the complexities of bone marrow aspiration, purification, and cell preparation [22,23]. Histologic evaluation of the hearts suggested there was less scar tissue in the PRP-treated hearts as compared to PBS-treated controls.

Although this study was not specifically designed to validate a mechanism for these findings, it is possible to speculate about potential reasons for the findings. It is well established that PRP contains highly concentrated growth factors and other bioactive substances [8]. Insulin-like growth factor-1 (IGF-1), present in PRP, has been shown to stimulate cell-protective mechanisms against oxidative stress and inflammation. It has also been found to protect cardiac progenitor cells (CPC) from death and promote proliferation of adult cardiomyocytes. IGF-1 has further been shown to enhance the migration of mesenchymal stem cells from bone marrow to an infarct site [24]. IGF-1 in combination with CPC therapy has been shown to improve recovery of myocardial structure and function after infarction [25]. RevaTen PRP contains a variety of other growth factors and concentrated white blood cells that may preserve or protect ischemic or damaged myocardial tissue. Several investigators have previously examined the role of various growth factors and inflammatory cells in myocardial injury and ischemia/reperfusion injury. Intramyocardial transplantation of fibroblasts expressing VEGF has been shown to attenuate cardiac dysfunction after myocardial infarction [26]. Basic FGF has also been shown to provide a protective effect against ischemia–reperfusion-induced oxidative damage, cell death, and infarction [27]. Monocytes have been shown to promote angiogenesis and myocyte survival in a model of myocardial infarction and also improve blood

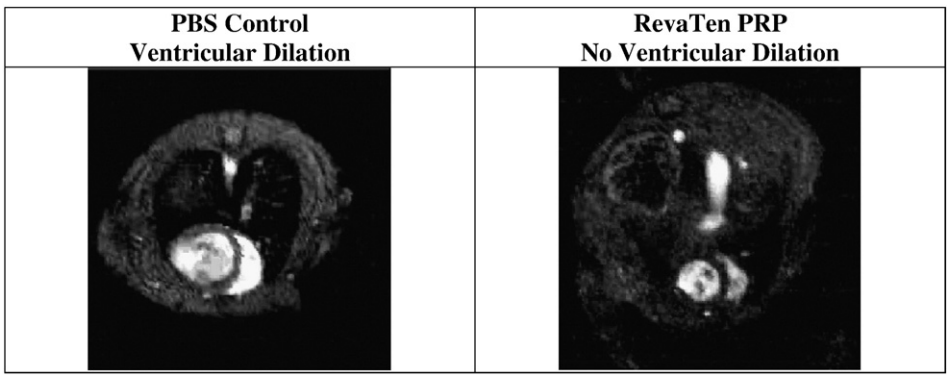

Fig. 3. Cardiac MRI of PBS control and RevaTen PRP.

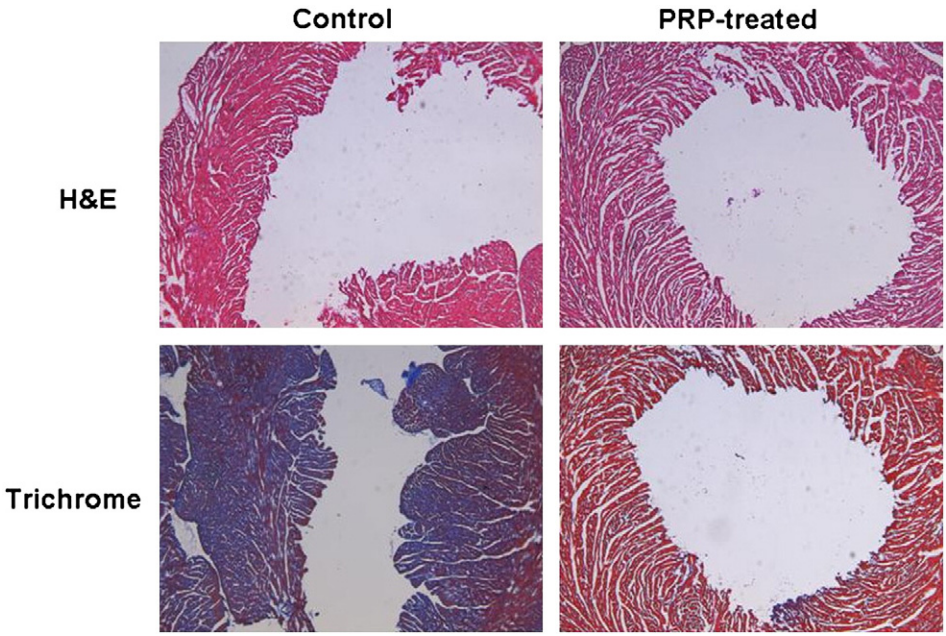

Fig. 4. Histology of control and RevaTen PRP-treated hearts.

perfusion and capillary density in hind limb ischemia model [28,29]. Finally, lymphocytes also participate in the healing response from ischemia–reperfusion injury [30,31].

RevaTen PRP may further act to help preserve cardiac fibroblasts and/or myocytes at risk for ischemic or apoptotic death via the release of growth factors and cytokines. Souders et al. [32] have demonstrated the importance of the cardiac fibroblast in maintaining the extracellular matrix and in providing a scaffold for myocytes to ensure proper heart form and function. If RevaTen PRP helps these cells survive and thrive after ischemia–reperfusion injury, negative remodeling could be limited. Although not examined in the present study, we believe that cardiac fibroblast stimulation by TGF-β within RevaTen PRP should result in increased synthesis of fibrillar collagen, proteoglycans, and expression of contractile genes. Importantly, FGF and VEGF (both present in RevaTen PRP) are crucial for enhancing angiogenesis and promoting collateral formation after myocardial injury. In addition, PRP may inhibit or facilitate known, or as yet unknown, signaling pathways.

This investigation is primarily an observational study and, as such, has inherent limitations. It was not designed to specifically elucidate a mechanism or set of mechanisms behind the functional findings. Further investigations will need to be designed to evaluate the mechanisms behind the marked improvement in cardiac function. These functional outcomes must also be better correlated with histologic findings and biologic markers using in vitro and in vivo trials. Importantly, the functional findings should also be directly compared to stem cell therapies. It may be possible that the cytokines are more important than the cells in the treatment of injured cardiac tissue.

Platelet cell therapy represents a paradigm shift in biologic therapy for treatment of injured myocardial tissues. The combination of cytokines from platelets with bioactive white blood cells can be obtained rapidly from whole blood at the point of care. PRP is an autologous product with an extensive clinical use history and an excellent safety record. This study strongly suggests intramyocardial injection of RevaTen PRP after myocardial injury results in improved LVEF. If function can be preserved after myocardial injury, it may be possible to limit the progression to congestive heart failure and thereby reduce medical costs. Further preclinical and clinical investigation into the uses of RevaTen PRP in repairing cardiac tissue, including elucidation of the optimal delivery, dosage, and formulation of PRP, is warranted.

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