The Effect of Platelet-Rich Plasma Formulations and Blood Products on Human Synoviocytes: Implications for Intra-articular Injury and Therapy

Hillary J. Braun, Hyeon Joo Kim, Constance R. Chu and Jason L. Dragoo

Additional services and information for *The American Journal of Sports Medicine* can be found at:

P<P

Published online March 14, 2014 in advance of the print journal.

Email Alerts: http://ajs.sagepub.com/cgi/alerts

Subscriptions: http://ajs.sagepub.com/subscriptions

Reprints: http://www.sagepub.com/journalsReprints.nav

Permissions: http://www.sagepub.com/journalsPermissions.nav

>> OnlineFirst Version of Record - Mar 14, 2014

What is This?

The Effect of Platelet-Rich Plasma Formulations and Blood Products on Human Synoviocytes

Implications for Intra-articular Injury and Therapy

Hillary J. Braun,*[†] BA, Hyeon Joo Kim,* PhD, Constance R. Chu,* MD, and Jason L. Dragoo,*[‡] MD
Investigation performed at Stanford University, Palo Alto, California, USA

Background: The effect of platelet-rich plasma (PRP) on chondrocytes has been studied in cell and tissue culture, but considerably less attention has been given to the effect of PRP on synoviocytes. Fibroblast-like synoviocytes (FLS) compose 80% of the normal human synovium and produce cytokines and matrix metalloproteinases that can mediate cartilage catabolism.

Purpose: To compare the effects of leukocyte-rich PRP (LR-PRP), leukocyte-poor PRP (LP-PRP), red blood cell (RBC) concentrate, and platelet-poor plasma (PPP) on human FLS to determine whether leukocyte and erythrocyte concentrations of PRP formulations differentially affect the production of inflammatory mediators.

Study Design: Controlled laboratory study.

Methods: Peripheral blood was obtained from 4 donors and processed to create LR-PRP, LP-PRP, RBCs, and PPP. Human synoviocytes were cultured for 96 hours with the respective experimental conditions using standard laboratory conditions. Cell viability and inflammatory mediator production were then evaluated.

Results: Treatment with LR-PRP resulted in significantly greater synoviocyte death (4.9% $\pm$ 3.1%) compared with LP-PRP (0.72% $\pm$ 0.70%; P = .035), phosphate-buffered saline (PBS) (0.39% $\pm$ 0.27%; P = .018), and PPP (0.26% $\pm$ 0.30%; P = .013). Synoviocytes treated with RBC concentrate demonstrated significantly greater cell death (12.5% $\pm$ 6.9%) compared with PBS (P < .001), PPP (P < .001), LP-PRP (P < .001), and LR-PRP (4.9% $\pm$ 3.1%; P < .001). Interleukin (IL)–1β content was significantly higher in cultures treated with LR-PRP (1.53 $\pm$ 0.86 pg/mL) compared with those treated with PBS (0.22 $\pm$ 0.295 pg/mL; P < .001), PPP (0.11 $\pm$ 0.179 pg/mL; P < .001), and RBCs (0.64 $\pm$ 0.58 pg/mL; P = .001). IL-6 content was also higher with LR-PRP (32,097.82 $\pm$ 22,844.300 pg/mL) treatment in all other groups (P < .001). Tumor necrosis factor–α levels were greatest in LP-PRP (9.97 $\pm$ 3.110 pg/mL), and this was significantly greater compared with all other culture conditions (P < .001). Interferon-γ levels were greatest in RBCs (64.34 $\pm$ 22.987 pg/mL) and significantly greater than all other culture conditions (P < .001).

Conclusion: Treatment of synovial cells with LR-PRP and RBCs resulted in significant cell death and proinflammatory mediator production.

Clinical Relevance: Clinicians should consider using leukocyte-poor, RBC-free formulations of PRP when administering intra-articularly.

Keywords: platelet-rich plasma; synoviocytes; osteoarthritis; inflammation

---

[‡]Address correspondence to Jason L. Dragoo, MD, Department of Orthopaedic Surgery, Stanford University, 450 Broadway Street, Pavilion C, 4th Floor, Redwood City, CA 94063-6342, USA (e-mail: jdragoo@stanford.edu).

*Department of Orthopaedic Surgery, Stanford University, Palo Alto, California, USA.

[†]School of Medicine, University of California–San Francisco, San Francisco, California, USA.

Platelet-rich plasma (PRP) is an autologous blood fraction composed of concentrated platelets and growth factors. The rationale behind PRP as a therapeutic agent is that it delivers growth factors, cytokines, and other molecular mediators to the site of tissue injury to increase healing. PRP is commonly used in clinical orthopaedics as an injection or gel biologic therapy to facilitate wound hemostasis[3,11] and to treat tendinopathies.[2,24,33] It has been used as an experimental treatment for osteoarthritis with mixed results.[9,21]

The effect of PRP on chondrocytes has been studied using in vitro and in vivo basic science models. A recent

systematic review of this literature demonstrated that 71% of the studies report PRP increases the synthetic capacity of chondrocytes, including upregulation of gene expression, proteoglycan production, and type II collagen deposition.[34] However, only 4.8% (1/21) of reports included in this analysis characterized the white blood cell (WBC), platelet, and red blood cell (RBC) contents of their PRP formulations.[39] There are many variations in PRP formulations, with both platelet and leukocyte concentrations having been identified as major constituents affecting the inflammatory responses after PRP injection.

Less attention has been given to the effect of PRP on synoviocytes. When considering intra-articular applications, it is imperative to assess the effect of the therapy on other cell populations within the joint. Fibroblast-like synoviocytes (FLS) compose 80% of the normal human synovium and produce cytokines and matrix metalloproteinases (MMPs) that can mediate cartilage catabolism. A recent study by Browning et al[4] found that, compared with platelet-poor plasma (PPP) and phosphate-buffered saline (PBS), PRP treatment resulted in increases of cytokines and proteases produced by human FLS. Their study used leukocyte-rich PRP (LR-PRP), which has been shown to cause an increased acute inflammatory response compared with leukocyte-poor PRP (LP-PRP).[7]

The purpose of this study was to compare the effects of LR-PRP, LP-PRP, and RBCs on human FLS to determine whether the addition of leukocytes and/or erythrocytes to PRP formulations affects FLS production of inflammatory mediators.

METHODS

Institutional review board approval was obtained before the initiation of this study.

Preparation of Blood Products

Approximately 110 mL blood was obtained by a licensed phlebotomist from 4 healthy volunteers without medical history using a standard venipuncture technique. In total, 55 mL was processed using the High-Yield PRP System (Emcyte Corporation, Fort Meyers, Florida, USA) according to the manufacturer's instructions to create LR-PRP, PPP, and packed RBCs. The remaining 55 mL was simultaneously processed using Pure PRP (Emcyte Corporation) to create LP-PRP, a formulation without neutrophils. The following blood product groups were analyzed: whole blood, PPP, LR-PRP, LP-PRP, and concentrated RBCs. Complete blood count (CBC) was used to confirm WBC, RBC, and platelet concentrations. Samples were stored at –80°C until they were applied to cell culture.

Synoviocyte Culture

Human type B FLS were obtained from a commercially available cell line (Cell Applications, San Diego, California, USA) and expanded in Synoviocyte Growth Media (Cell Applications). Synoviocytes were seeded at a density of 1.3×10^5 cells/well. Three wells were used per culture condition; each contained media and 3% blood/PRP product depending on the experimental group. Cells were cultured for 96 hours before viability and cytokine analysis was performed.

Live/Dead Viability Analysis

A Live/Dead Viability/Cytotoxicity Assay (Invitrogen Corporation, Carlsbad, California, USA), a 2-color fluorescence assay, was used for staining of the cultures after a respective 96-hour culture. Probes calcein AM and ethidium homodimer (EthD-1) were used. Cells were stained at room temperature for 30 minutes in the dark. The ratio of dead to live cells was assessed by fluorescent microscopy. Microscopic analysis was completed immediately after incubation using a Zeiss Observer Z1 (Carl Zeiss, Inc, Thornwood, New York, USA) fluorescent microscope. Images were taken using an AxioCam MRm camera (Carl Zeiss). Two frames were taken using the GP and DsRED2 filters, wavelengths 510 ± 10 and 590 ± 14, respectively, and were overlaid to produce a single image. Four random high-powered fields (HPFs; 100×) were acquired for each well. Automated cell-counting parameters were set based on the size of the smallest and largest cells visualized. Manual counting was performed to validate automated cell-counting parameters. Manual and automated counts were equal to ±4 cells. The ratio of dead to live cells was then calculated.

Cytokine Analysis

To evaluate proinflammatory mediator production, cell culture supernatant was analyzed for the following panel of anabolic and catabolic cytokines and molecular mediators, which are known to play an active role in both the initiation and response to joint tissue injury: interleukin (IL)–1β, IL-4, IL-6, IL-10, interferon (IFN)–γ, and tumor necrosis factor (TNF)–α using a Bio-Rad Multiplex analysis (Bio-Plex, Bio-Rad, Hercules, California, USA) according to the manufacturer's protocol.

Statistical Analysis

All statistical analyses were performed with SPSS Version 21 (SPSS, Inc, an IBM Company, Chicago, Illinois, USA). Descriptive statistics for platelet, WBC, and RBC counts were calculated for the blood products obtained from the 4 donors. For cell viability, the results of the 4 HPFs per well were averaged and the 3 wells per condition were compared with all other conditions using a 1-way analysis of variance (ANOVA) with Bonferroni post hoc correction. Cytokine results were also analyzed using a 1-way ANOVA with Bonferroni correction. Significance was set at $P < .05$.

RESULTS

Blood Products

Both LR-PRP and LP-PRP were defined as having a concentration of platelets greater than that in whole blood.

TABLE 1
Summary of Blood Product Data[a]

Donor	Whole Blood–Plt	Whole Blood–WBC	Whole Blood–RBC	LR-PRP–Plt	LR-PRP–WBC	LR-PRP–RBC	LP-PRP–Plt	LP-PRP–WBC	LP-PRP–RBC	PPP-Plt	PPP-WBC	PPP-RBC	RBCC-Plt	RBCC-WBC	RBCC-RBC
1	173	7.3	4.84	510	23.8	1.27	414	4.4	0.12	30	0.1	0.01	20	1.6	9.9
2	262	7.3	5.05	1071	44.5	7.14	1136	7.3	0.11	41	0	0.01	18	1.3	10.1
3	225	9.4	4.13	747	12.6	4.35	629	8	0.04	20	0.1	0.01	4	0.5	7.65
4	176	4.6	5.18	882	25.5	2.31	779	4.7	0.06	20	0.1	0	42	3.1	10.87
Avg	209	7.15	4.8	802.5	26.6	3.7675	739.5	6.1	0.0825	27.75	0.075	0.0075	21	1.625	9.63

[a]Individual and averaged data for blood products used in synoviocyte culture. Avg, average; LR, leukocyte rich; LP, leukocyte poor; Plt, platelet; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell; RBCC, red blood cell concentrate; WBC, white blood cell. Plt and WBC units in K/μL; RBC units in MIL/μL.

TABLE 2
Average and Range of Blood Product Data[a]

	Whole Blood	LR-PRP	LP-PRP	PPP	RBCC
Plt, K/μL	209 (173-262)	802.5 (510-1071)	740 (414-1136)	27.8 (20-41)	21 (4-42)
WBC, K/μL	5.9 (4.3-7.3)	26.6 (12.6-44.5)	6.1 (4.4-8)	0.08 (0-0.1)	1.6 (0.5-3.1)
RBC, MIL/μL	4.8 (4.13-5.18)	3.8 (1.27-7.14)	0.09 (0.04-0.12)	0.008 (0-0.01)	9.6 (7.7-10.9)

[a]Avg, average; LR, leukocyte rich; LP, leukocyte poor; Plt, platelet; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell; RBCC, red blood cell concentrate; WBC, white blood cell.

LR-PRP was defined as having an overall WBC count greater than the WBC count in whole blood, while LP-PRP was defined as having an overall WBC count less than the WBC count in whole blood. Platelet-poor plasma was characterized by a concentration of platelets less than that in whole blood, and RBC concentrate contained a greater concentration of platelets than whole blood. Individual and average CBC data for whole blood, LR-PRP, LP-PRP, PPP, and RBC concentrate are summarized in Table 1. Average and range data are condensed in Table 2.

Baseline Values

Cytokine levels at baseline and 96 hours for PPP, LP-PRP, LR-PRP, and RBCs are compared in Table 3. In PPP, levels of IL-6, IL-10, and TNF-α were significantly greater at 96 hours versus baseline. In LP-PRP, IL-1β, IL-6, and IL-10 were significantly greater at 96 hours compared with baseline. IL-1β and IL-6 were significantly greater in LR-PRP at 96 hours versus baseline. Finally, IL-6 and IFN-γ were significantly greater in RBCs at 96 hours compared with baseline.

LR-PRP

Treatment with LR-PRP resulted in significantly greater cell death (4.9% $\pm$ 3.1%) compared with LP-PRP (0.72% $\pm$ 0.70%; P = .035), PBS (0.39% $\pm$ 0.27%; P = .018), and PPP (0.26% $\pm$ 0.30%; P = .013). After 96 hours of culture, IL-1β content was significantly higher in cultures treated with LR-PRP (1.53 $\pm$ 0.86 pg/mL) compared with those treated with PBS (0.22 $\pm$ 0.295; P < .001), PPP (0.11 $\pm$

0.179; P < .001), and RBCs (0.64 $\pm$ 0.58; P = .001) (Table 3). There was no difference between the IL-1β content of LR-PRP versus LP-PRP (0.68 $\pm$ 0.56; P = .097). Similarly, IL-6 content was higher in LR-PRP–treated groups (32,097.82 $\pm$ 22,844.300 pg/mL) compared with PBS (5426.00 $\pm$ 3421.896 pg/mL; P < .001), PPP (1689.96 $\pm$ 773.026 pg/mL; P < .001), and RBCs (6794.45 $\pm$ 5027.470 pg/mL; P < .001). There was no difference between the IL-6 content of LR-PRP versus LP-PRP (15,388.3 $\pm$ 11,826.2; P = .531). IL-4 content was significantly greater in LR-PRP–treated groups (2.90 $\pm$ 3.8 pg/mL) compared with PBS (0 $\pm$ 0 pg/mL; P < .001), PPP (0 $\pm$ 0 pg/mL; P < .001), and RBCs (0.22 $\pm$ 0.87 pg/mL; P < .001). There was no difference in IL-4 content between LR-PRP and LP-PRP (3.61 $\pm$ 3.31 pg/mL; P = 1.0).

LP-PRP

LP-PRP treatment resulted in significantly less cell death (0.72% $\pm$ 0.7%) than RBC treatment (12.5% $\pm$ 6.9%; P < .001) and LR-PRP treatment (4.9% $\pm$ 3.1%; P = .035). TNF-α levels were greatest in LP-PRP (9.97 $\pm$ 3.110 pg/mL), and this was significantly greater compared with PBS (0.34 $\pm$ 487 pg/mL; P < .001), LR-PRP (3.36 $\pm$ 1.943 pg/mL; P < .001), and RBCs (1.60 $\pm$ 688 pg/mL; P < .001) (Table 3). IL-4 levels were significantly greater in LP-PRP (3.61 $\pm$ 3.3 pg/mL) compared with PBS (0 $\pm$ 0; P < .001), PPP (0 $\pm$ 0; P < .001), and RBCs (0.22 $\pm$ 0.87; P < .001). IL-10 levels were also significantly greater after LP-PRP treatment (14.04 $\pm$ 14.5) versus PBS (0 $\pm$ 0; P = .005), LR-PRP (1.95 $\pm$ 3.1; P = .021), and RBCs (0 $\pm$ 0; P =

TABLE 3
Summary of Baseline and 96-Hour Cytokine Data[a]

	IL-1β	IL-4	IL-6	IL-10	IFN-γ	TNF-α
PPP						
0-hour	0 ± 0	0 ± 0	0 ± 0	1.0 ± 2.0	23.3 ± 26.1	2.1 ± 0.9
96-hour	0.11 ± 0.18	0 ± 0	1690.0 ± 773.0	30.4 ± 15.5	9.5 ± 7.0	8.9 ± 5.1
P value	.264	NA	**.001**	**.002**	.100	**.020**
LP-PRP						
0-hour	0 ± 0	6.3 ± 5.0	0 ± 0	0 ± 0	47.2 ± 43.3	10.2 ± 4.0
96-hour	0.9 ± 0.5	2.7 ± 2.1	20,517.7 ± 8712.4	18.7 ± 13.8	21.2 ± 8.3	10.0 ± 3.1
P value	**.002**	.058	**.000**	**.019**	.054	.909
LR-PRP						
0-hour	0.3 ± 0.2	2.6 ± 4.2	0.4 ± 0.8	0 ± 0	91.2 ± 73.9	6.1 ± 2.6
96-hour	1.53 ± 0.9	3.0 ± 3.8	32,097.8 ± 22,844.3	2.6 ± 3.4	35.5 ± 17.4	3.4 ± 1.9
P value	**.013**	.847	**.016**	.154	**.022**	**.041**
RBC						
0-hour	0.3 ± 0.5	0 ± 0	0 ± 0	0 ± 0	31.4 ± 37.0	1.5 ± 0.5
96-hour	0.6 ± 0.6	0.3 ± 1.0	6794.5 ± 5027.5	0 ± 0	64.3 ± 23.0	1.6 ± 0.6
P value	.348	.582	**.019**	NA	**.050**	.794

[a]Levels of interleukin (IL)–1β, IL-4, IL-6, IL-10, interferon (IFN)–γ, and tumor necrosis factor (TNF)–α at baseline and 96 hours for platelet-poor plasma (PPP), leukocyte-poor (LP)–platelet-rich plasma (PRP), leukocyte-rich (LR)–PRP, and red blood cells (RBCs). Bolded values indicate statistically significant difference ($P \leq .05$). NA indicates no statistical tests were performed because concentration was 0 at both time points.

.004). There was no significant difference in IL-10 levels between LP-PRP and PPP (23.03 ± 18.7; *P* = .204).

Platelet-Poor Plasma

Platelet-poor plasma treatment resulted in significantly less cell death (0.26% ± 0.3%) versus RBC treatment (12.5% ± 6.9%; *P* < .001) and LR-PRP treatment (4.9% ± 3.1%; *P* = .013). TNF-α levels in PPP (7.18 ± 5.3 pg/mL) were significantly greater than those in PBS (0.34 ± 487; *P* < .001), LR-PRP (3.36 ± 1.943; *P* = .043), and RBCs (1.60 ± 688; *P* < .001). There was no significant difference compared with LP-PRP (9.97 ± 3.110; *P* = .095). IL-10 levels were also significantly greater in PPP (23.03 ± 18.7 pg/mL) versus PBS (*P* < .001), LR-PRP (*P* < .001), and RBCs (*P* < .001). There was no significant difference compared with LP-PRP (14.04 ± 14.5; *P* = .204).

RBC Concentrate

Synoviocytes treated with RBC concentrate demonstrated significantly greater cell death (12.5% ± 6.9%) compared with PBS (*P* < .001), PPP (*P* < .001), LP-PRP (*P* < .001), and LR-PRP (4.9% ± 3.1%; *P* < .001) (Figure 1). IFN-γ levels were greatest in RBCs (64.34 ± 22.987 pg/mL) and significantly greater than all other culture conditions (*P* < .001) (Table 3). Red blood cell treatment did not result in the production of a significant amount of anti-inflammatory cytokines (IL-4, 0.22 ± 0.87 pg/mL; IL-10, 0 ± 0 pg/mL).

DISCUSSION

The purpose of this study was to assess the effect of LR-PRP, LP-PRP, and RBCs on the viability and
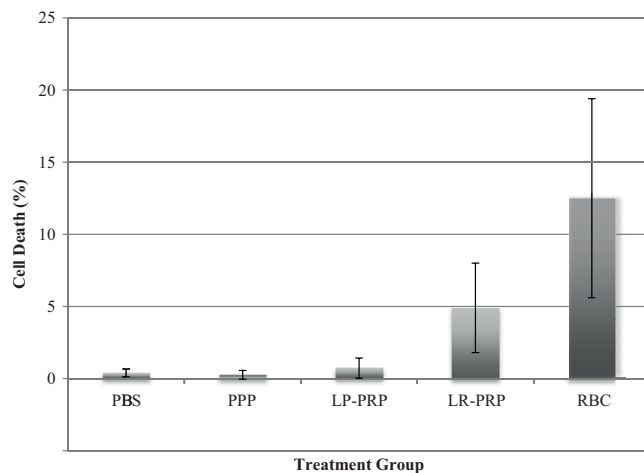

Figure 1. Average synoviocyte death for each condition after 96 hours of cell culture. Red blood cell treatment resulted in significantly greater cell death compared with all other culture conditions (*P* < .001). LR, leukocyte rich; LP, leukocyte poor; PBS, phosphate-buffered saline; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell.

inflammatory mediator production of human synoviocytes. Our results demonstrated that LR-PRP and RBC treatment resulted in significant synoviocyte death compared with LP-PRP and controls. Treatment with LR-PRP, LP-PRP, and RBCs resulted in significantly increased production of proinflammatory mediators. Anti-inflammatory mediators were also significantly increased in cultures treated with LR-PRP, LP-PRP, and PPP.

Normal synovium is composed of 2 morphologically distinct cell types: phagocytotic, macrophage-like synoviocytes and FLS. Fibroblast-like synoviocytes play a largely

homeostatic role via the production of the extracellular matrix and secretion of hyaluronan and lubricin.[14,15,25] Fibroblast-like synoviocytes can be activated (injured) through a variety of pathways, including stimulation by cytokines, chemokines, growth factors, and bioactive lipids produced by infiltrating leukocytes or other FLS.[27] Activation overrides the homeostatic function of FLS and instead promotes the production of proinflammatory mediators and degradative enzymes. Downstream effects of activation include synovial hyperplasia, increased synthesis of proinflammatory mediators, and destruction of cartilage extracellular matrix.[27]

Platelet-rich plasma is commonly injected into tendons and ligaments in the upper and lower extremities, but intra-articular injections of PRP have been relatively limited to the treatment of knee osteoarthritis (OA). In the past 5 years, clinical studies have demonstrated conflicting results on the efficacy of PRP for treatment of OA.[§] Older, nonrandomized controlled trials consistently reported significant improvements in clinical pain and function measurements after multiple intra-articular injections of PRP.[9,12,21,22,31,32,39] While these results initially appeared encouraging, many of these studies did not account for, or document, the leukocyte or erthryocyte concentrations of PRP preparations.

Recent work from 2012 and 2013 has increasingly emphasized the importance of characterizing the contents of PRP formulations. The findings in our basic science study that LR-PRP resulted in significantly greater cell death and synoviocyte activation compared with LP-PRP are relevant when choosing clinical treatments for intra-articular lesions. All 4 major proinflammatory cytokines (IL-1β, IL-6, IFN-γ, and TNF-α) were significantly increased in synoviocyte cultures treated with LR-PRP (Table 4). In addition to the significant postinjection pain reported after intra-articular LR-PRP treatment,[22] it is established that these cytokines incite and perpetuate inflammation and contribute to subsequent cartilage degradation[5,40] and, most significantly, that overexpression of TNF-α, IL-1β, and IL-6 is sufficient to induce arthritis.[1,17,20] Furthermore, LR-PRP and LP-PRP caused a significant production of the anti-inflammatory mediator IL-4, with LP-PRP causing the greatest amount of IL-4 and a significantly greater amount of IL-10. Both IL-4 and IL-10 are potent anti-inflammatory mediators that individually and synergistically reduce the production of IL-1β and TNF-α.[41,42] The results of the present study, in the context of previous clinical and basic science findings, support a transition away from LR-PRP and toward LP-PRP in the clinical context for intra-articular use; LP-PRP activates fewer proinflammatory cytokines and more anti-inflammatory cytokines while still delivering an increased concentration of platelets to the site of injury.

An additional pertinent finding is the deleterious effect of RBCs on the synovium. Hemarthrosis is observed in >90% of patients with joint trauma who have surgically significant intra-articular lesions,[6] and it has been

---

§References 9, 10, 12, 13, 19, 21, 22, 28, 31, 32, 35, 37, 39.

TABLE 4
Blood Product Cytokines[a]

	IL-1β	IL-6	IFN-γ	TNF-α	IL-4	IL-10
PPP				+		+
LP-PRP				+	+	+
LR-PRP	+	+	+	+	+	
RBC			+			

[a]Comparison of cytokine concentration versus phosphate-buffered saline (PBS) control. +Significantly greater than PBS control ($P < .05$). IFN, interferon; IL, interleukin; LR, leukocyte rich; LP, leukocyte poor; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RBC, red blood cell; TNF, tumor necrosis factor.

postulated that the presence of intra-articular blood may induce cartilage damage directly via iron-catalyzed formation of reactive oxygen species[16,29,30,36] and indirectly via hemosiderin-induced activation of the synovium.[26,38] The results of our study indicate that synoviocytes are not only killed by RBCs but also stimulated to release catabolic mediators such as IFN-γ and members of the IL-1 family. In posttraumatic OA specifically, increased levels of IL-1, IL-6, and TNF-α correlate with the severity of cartilage damage and inflammatory response after acute injury.[5,8,18,23,40] Furthermore, multiple studies have demonstrated that administration of IL-4 and IL-10 can protect against blood-induced joint injury.[41,42] The synoviocytes treated with RBC concentrate in our study did not produce a significant amount of either of these anti-inflammatory mediators. In LR-PRP– and LP-PRP–treated groups, where cell death was less extensive than in the RBC-treated group, IL-4 and IL-10 production increased in response to the insult. Our findings, in conjunction with the established pathways of intra-articular inflammation and degradation, indicate that the presence of RBCs may lead to degenerative changes within the joint through the death of synoviocytes, the direct increase of catabolic mediators, and the failure to respond with increased levels of crucial anti-inflammatory mediators. It appears that caution should be taken to avoid administering RBCs intra-articularly, which may have implications for clinical treatments such as PRP injection, microfracture, and timely aspiration after joint trauma.

There are several limitations of this study. First, monolayer synoviocytes were analyzed instead of intact synovial specimens. An intact tissue specimen, which would be present in vivo, may afford some protection to the cells that was not appreciated in this investigation. Second, this in vitro model is not equivalent to in vivo conditions; our design could only approximate the true joint environment. Finally, samples were analyzed at a single time point (96 hours), and further studies may reveal different effects at both earlier and later time points. Despite these limitations, our results provide evidence that intra-articular administration of blood products containing a concentrated amount of leukocytes or RBCs may be toxic to synoviocytes and should be used with caution. These findings further underscore the need to exercise caution when administering any substance intra-articularly.

CONCLUSION

The results of this study demonstrate that LR-PRP and RBCs are particularly cytotoxic to synoviocytes. Both treatments resulted in significant synoviocyte death compared with LP-PRP, PPP, and PBS. LR-PRP treatment caused a significant increase in proinflammatory cytokines IL-1β and IL-6, while RBC treatment caused significant production of IFN-γ and LP-PRP treatment caused significant production of TNF-α. These results appear to suggest that the interaction of either (a) polymorphonuclear WBCs or (b) erythrocytes with synoviocytes results in cell activation, release of proinflammatory mediators, or death. Whether due to an injection of LR-PRP or an intra-articular joint bleed (RBCs), it is important to recognize that these substances may activate inflammation and lead to synovial injury.

REFERENCES

1. Atsumi T, Ishihara K, Kamimura D, et al. A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. *J Exp Med*. 2002;196(7):979-990.
2. Barrett SL, Erredge SE. Growth factors for chronic plantar fasciitis. *Podiatry Today*. 2004;17:37-42.
3. Berghoff WJ, Pietrzak WS, Rhodes RD. Platelet-rich plasma application during closure following total knee arthroplasty. *Orthopedics*. 2006;29(7):590-598.
4. Browning SR, Weiser AM, Woolf N, et al. Platelet-rich plasma increases matrix metalloproteinases in cultures of human synovial fibroblasts. *J Bone Joint Surg Am*. 2012;94(23):e1721-e1727.
5. Chandrasekhar S, Harvey AK, Hrubey PS. Intra-articular administration of interleukin-1 causes prolonged suppression of cartilage proteoglycan synthesis in rats. *Matrix*. 1992;12(1):1-10.
6. DeHaven KE. Diagnosis of acute knee injuries with hemarthrosis. *Am J Sports Med*. 1980;8(1):9-14.
7. Dragoo JL, Braun HJ, Durham JL, et al. Comparison of the acute inflammatory response of two commercial platelet-rich plasma systems in healthy rabbit tendons. *Am J Sports Med*. 2012;40(6):1274-1281.
8. Elsaid KA, Fleming BC, Oksendahl HL, et al. Decreased lubricin concentrations and markers of joint inflammation in the synovial fluid of patients with anterior cruciate ligament injury. *Arthritis Rheum*. 2008;58(6):1707-1715.
9. Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. *Knee Surg Sports Traumatol Arthrosc*. 2011;19(4):528-535.
10. Filardo G, Kon E, Di Martino A, et al. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: study design and preliminary results of a randomized controlled trial. *BMC Musculoskelet Disord*. 2012;13:229.
11. Gardner MJ, Demetrakopoulos D, Klepchick PR, Mooar PA. The efficacy of autologous platelet gel in pain control and blood loss in total knee arthroplasty: an analysis of the haemoglobin, narcotic requirement and range of motion. *Int Orthop*. 2007;31(3):309-313.
12. Gobbi A, Karnatzikos G, Mahajan V, Malchira S. Platelet-rich plasma treatment in symptomatic patients with knee osteoarthritis: preliminary results in a group of active patients. *Sports Health*. 2012;4(2):162-172.
13. Halpern B, Chaudhury S, Rodeo SA, et al. Clinical and MRI outcomes after platelet-rich plasma treatment for knee osteoarthritis. *Clin J Sport Med*. 2013;23(3):238-239.
14. Helder AE, Feltkamp-Vroom TM, Nienhuis RL. Electron and light microscopical observations and serological findings in rheumatoid arthritis. *Ann Rheum Dis*. 1973;32(6):515-523.
15. Henderson B, Pettipher ER. The synovial lining cell: biology and pathobiology. *Semin Arthritis Rheum*. 1985;15(1):1-32.
16. Hooiveld M, Roosendaal G, Vianen M, van den Berg M, Bijlsma J, Lafeber F. Blood-induced joint damage: long-term effects in vitro and in vivo. *J Rheumatol*. 2003;30(2):339-344.
17. Horai R, Saijo S, Tanioka H, et al. Development of chronic inflammatory arthropathy resembling rheumatoid arthritis in interleukin 1 receptor antagonist-deficient mice. *J Exp Med*. 2000;191(2):313-320.
18. Irie K, Uchiyama E, Iwaso H. Intraarticular inflammatory cytokines in acute anterior cruciate ligament injured knee. *Knee*. 2003;10(1):93-96.
19. Jang SJ, Kim JD, Cha SS. Platelet-rich plasma (PRP) injections as an effective treatment for early osteoarthritis. *Eur J Orthop Surg Traumatol*. 2013;23(5):573-580.
20. Keffer J, Probert L, Cazlaris H, et al. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J*. 1991;10(13):4025-4031.
21. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sports Traumatol Arthrosc*. 2010;18(4):472-479.
22. Kon E, Mandelbaum B, Buda R, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: from early degeneration to osteoarthritis. *Arthroscopy*. 2011;27(11):1490-1501.
23. Marks PH, Donaldson ML. Inflammatory cytokine profiles associated with chondral damage in the anterior cruciate ligament–deficient knee. *Arthroscopy*. 2005;21(11):1342-1347.
24. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med*. 2006;34(11):1774-1778.
25. Mor A, Abramson SB, Pillinger MH. The fibroblast-like synovial cell in rheumatoid arthritis: a key player in inflammation and joint destruction. *Clin Immunol*. 2005;115(2):118-128.
26. Morris CJ, Blake DR, Wainwright AC, Steven MM. Relationship between iron deposits and tissue damage in the synovium: an ultrastructural study. *Ann Rheum Dis*. 1986;45(1):21-26.
27. Noss EH, Brenner MB. The role and therapeutic implications of fibroblast-like synoviocytes in inflammation and cartilage erosion in rheumatoid arthritis. *Immunol Rev*. 2008;223:252-270.
28. Patel S, Dhillon MS, Aggarwal S, Marwaha N, Jain A. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: a prospective, double-blind, randomized trial. *Am J Sports Med*. 2013;41(2):356-364.
29. Roosendaal G, Vianen ME, Marx JJ, van den Berg HM, Lafeber FP, Bijlsma JW. Blood-induced joint damage: a human in vitro study. *Arthritis Rheum*. 1999;42(5):1025-1032.
30. Roosendaal G, Vianen ME, van den Berg HM, Lafeber FP, Bijlsma JW. Cartilage damage as a result of hemarthrosis in a human in vitro model. *J Rheumatol*. 1997;24(7):1350-1354.
31. Sampson S, Reed M, Silvers H, Meng M, Mandelbaum B. Injection of platelet-rich plasma in patients with primary and secondary knee osteoarthritis: a pilot study. *Am J Phys Med Rehabil*. 2010;89(12):961-969.
32. Sanchez M, Anitua E, Azofra J, Aguirre JJ, Andia I. Intra-articular injection of an autologous preparation rich in growth factors for the treatment of knee OA: a retrospective cohort study. *Clin Exp Rheumatol*. 2008;26(5):910-913.
33. Scioli M. Treatment of recalcitrant enthesopathy of the hip with platelet rich plasma—a report of three cases. *Clin Orthop Soc News*. 2006;(Spring):6-7.
34. Smyth NA, Murawski CD, Fortier LA, Cole BJ, Kennedy JG. Platelet-rich plasma in the pathologic processes of cartilage: review of basic science evidence. *Arthroscopy*. 2013;29(8):1399-1409.

35. Spakova T, Rosocha J, Lacko M, Harvanova D, Gharaibeh A. Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. *Am J Phys Med Rehabil*. 2012;91:411-417.

36. Stein H, Duthie RB. The pathogenesis of chronic haemophilic arthropathy. *J Bone Joint Surg Br*. 1981;63(4):601-609.

37. Torrero JI, Aroles F, Ferrer D. Treatment of knee chondropathy with platelet rich plasma: preliminary results at 6 months of follow-up with only one injection. *J Biol Regul Homeost Agents*. 2012;26(2)(suppl 1):71S-78S.

38. Valentino LA, Hakobyan N, Kazarian T, Jabbar KJ, Jabbar AA. Experimental haemophilic synovitis: rationale and development of a murine model of human factor VIII deficiency. *Haemophilia*. 2004;10(3):280-287.

39. van Buul GM, Koevoet WL, Kops N, et al. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. *Am J Sports Med*. 2011;39(11):2362-2370.

40. van den Berg WB, Joosten LA, van de Loo FA. TNF alpha and IL-1 beta are separate targets in chronic arthritis. *Clin Exp Rheumatol*. 1999;17(6)(suppl 18):S105-S114.

41. van Meegeren ME, Roosendaal G, Coeleveld K, Nieuwenhuizen L, Mastbergen SC, Lafeber FP. A single intra-articular injection with IL-4 plus IL-10 ameliorates blood-induced cartilage degeneration in haemophilic mice. *Br J Haematol*. 2013;160(4):515-520.

42. van Meegeren ME, Roosendaal G, Jansen NW, et al. IL-4 alone and in combination with IL-10 protects against blood-induced cartilage damage. *Osteoarthritis Cartilage*. 2012;20(7):764-772.