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Intra-Articular Injections of a Whole Blood Clot Secretome, Autologous Conditioned Serum, Have Superior Clinical and Biochemical Efficacy Over Platelet-Rich Plasma and Induce Rejuvenation-Associated Changes of Joint Metabolism: A Prospective, Controlled Open-Label Clinical Study in Chronic Knee Osteoarthritis

Larisa Shirokova,[1] Sergey Noskov,[1] Victoria Gorokhova,[1] Julio Reinecke,[2] and Ksenia Shirokova[1]

Abstract

Osteoarthritis is a frequent, age-associated disease affecting >10% of world's population over 60 years of age. This study intended to compare intra-articular whole blood clot secretome (autologous conditioned serum [ACS], recently re-named blood clot secretome [BCS]) to platelet-rich plasma (PRP) in knee osteoarthritis (OA). A clinical, nonrandomized open-label comparison of ACS versus PRP in knee OA with subclinical or moderate synovitis symptomology was performed. One hundred and twenty-three patients with knee OA, Kellgren and Lawrence grade II–III, were each treated with six i.a. injections of ACS or PRP. The clinical efficacy was measured by visual analog scale and Western Ontario and McMaster Universities Arthritis Index (WOMAC) score. The biochemical effects measured include synovial fluid (SF) viscosity, cytokines interleukin (IL)-1Ra and IL-1b, radical footprint NO_3, and conjugated dienes (CDs). At the 3-month follow-up, clinical efficacy of ACS was significant in all groups, versus PRP. PRP had significant versus baseline efficacy in subclinical, but not in moderate, synovitis cases. ACS was more effective than PRP regarding all analytical parameters. It induced endogenous IL-1Ra expression, downregulated IL-1b, and improved SF viscosity. ACS reduced—significantly stronger than PRP—the concentration of CDs—interpreted as reactive oxygen species footprints—and NO_3—interpreted as nitric oxide footprint—in SF. ACS displayed significant efficacy in all groups, which was clinically and biochemically superior to PRP. ACS appears to improve i.a. homeostasis. Strength of this open clinical study is the combination of clinical and biochemical data.

Keywords: osteoarthritis, clinical study, blood clot secretome, BCS, platelet-rich plasma, PRP, autologous conditioned serum, ACS

Introduction

Approximately >10% of the worldwide adult population >60 years of age are affected by osteoarthritis (OA), identifying it as one of the major health issues today.[1] OA is associated with aging and a disturbed balance of cartilage anabolism/catabolism. Signs of disease include cartilage destruction, bone alterations, reduced synovial fluid (SF) viscosity and effusion, excess oxygen radicals (reactive oxygen species [ROS]) and nitric oxide (NO), altered cytokine balance, cellular abnormalities, and synovial and cartilage tissue alterations such as hypertrophy and synovitis. OA pathology has also been linked to metabolic stress, insufficient nutrient availability, and impaired stem cell vitality.[2] An effective therapy for knee OA should thus address clinical (e.g., pain and function) and

[1]Department of Hospital Therapy, Yaroslavl State Medical University of MoH of RF, Yaroslavl, Russia.
[2]ORTHOGEN AG, Dusseldorf, Germany.

biochemical (e.g., inflammation and metabolic and energetic derailment) parameters.

In the past 10–20 years, biological therapies such as autologous treatments have gained increased attention. One reason for the appeal of autologous procedures is that the patient's own body provides the medication. Autologous conditioned serum (ACS), platelet-rich plasma (PRP), and preparations containing mesenchymal stromal cells (MSC) have therefore been the topic of numerous studies.

In this study, the authors evaluated two therapeutic techniques in a clinical study: PRP and ACS. The device for processing ACS was originally branded ''Orthokine'' and developed by Wehling and Reinecke in the late 1990s.[3] ACS was recently re-named blood clot secretome (BCS). ACS and PRP derive their active ingredients from the patient's blood. They contain signaling components, including growth factors (GF e.g., hepatocyte growth factor [HGF], platelet-derived growth factor [PDGF], transforming growth factor beta [TGFb], and insulin-like growth factor [IGF]-1) and cytokines (e.g., interleukin [IL]-1Ra and IL-10), potentially beneficial in OA. They both also contain vascular endothelial growth factor (VEGF) and many more possibly controversial GF. VEGF may induce capillary formation and PDGF may act inflammatory. All these factors will be present in the joint only for a limited time; it is therefore generally not perceived as a clinical risk. In PRP, these factors are packaged in alpha granules and require platelet degranulation to be released. In ACS, all factors are present as solute components. Both techniques are used in individual site-of-care procedures and are intended to alter the state of the joint from *de*generative to *re*generative.

ACS secretome is generated by coagulating blood under time and temperature control.[3–5] Secretomes are the sum of all substances released by cells.[6] Analogous to ACS, secretomes of MSC are deemed, in part, responsible for the therapeutic effects attributed to transplanted MSC.[7]

ACS demonstrated safety and efficacy in OA[8,9] and degenerative, traumatic, and inflammatory indications, including nerve,[10,11] muscle,[12,13] and tendon pathologies.[14–16] Postoperative treatment with ACS significantly reduced anterior cruciate ligament (ACL)-plasty-caused bone wasting (tunnel widening).[17] Adipose tissue-derived human MSC treated with pooled allogeneic ACS *in vitro* showed higher cell division and improved chondrocytic differentiation and immune-modulatory effects than their controls.[18]

PRP is a cell therapy with anticoagulated blood plasma harboring variably enhanced platelet counts obtained by mechanical separation. Meta-analyses cautiously review its clinical efficacy, often from ''limited'' to ''good.''[19–22]

This study compared the changes of clinical and biomarkers in knee OA patients treated with ACS or PRP. Ethical committee approval was obtained from Yaroslavl State Medical University. The permission for blood processing was granted by the Federal Service for Supervision of Health and Social Development for the application of new medical technology.

Methods

Biochemical analysis

SF viscosity was determined by capillary viscometry measuring the flow rate of the liquid in a capillary. For relative measurements, the viscosity-dependent value is compared to the same procedure for known viscosity fluids. In this study, the liquid with known viscosity was distilled water.[23]

SF concentration of IL-1Ra was determined by enzyme-linked immunosorbent assay (ELISA; BioSource Europe S.A., Nivelles, Belgium). SF concentration of IL-1b was determined by ELISA (Bender MedSystems, Austria). SF concentration of IL-6 was determined by ELISA (Vector-Best, Novosibirsk, Russia). SF concentrations of IGF-1 and TGF-b1 were determined externally by the independent diagnostic laboratory ''INVITRO LLC,'' Moscow.

SF NO_3 was measured with a nitrate ion-selective electrode with a potentiometer EV-74.[24] NO_3 was interpreted as a derivate of NO load.

Conjugated dienes (CDs) were used as measurement of lipid peroxidation by spectrophotometry at 231–234 nm. CDs are carbon double bonds interspaced by a single bond, regularly found in fatty acid residues, such as membrane lipids, after ROS attack.[25] High absorption was interpreted as a sign of high ROS load.

Processing of ACS and PRP

ACS was processed as described earlier.[3,8] Briefly, 40 mL of fresh whole patient blood was stored in the presence of medical grade borosilicate glass spheres at physiological temperature (24 hours at 37°C). Subsequently, ACS was separated from the blood clot by centrifugation for 10 minutes at 1670 g at room temperature (RT), passed through a 0.22 μm syringe tip filter and aliquoted, and stored at –20°C until use. A random sample of 21 ACS preparations was tested by ELISA for IL-1Ra, IL-1b, IL-6, IGF-1, and TGF-b1 (Table 1).

No commercial product for PRP processing was used. Instead, PRP was generated from 25 mL anticoagulated venous blood taken immediately before processing into disposable sterile syringes by use of a laboratory protocol. The blood was centrifuged for 9 minutes at 1670 g at RT. The middle layer of plasma supernatant was extracted (5 mL of 25 mL of blood). The lower layer of red blood cells and the upper layer of plasma were discarded. The number of platelets in the resulting PRP was $1–2.5 \times 10^9$ per mL, equivalent to approximately six to seven times the enrichment over baseline. No

Table 1. Cytokine- and Growth Factor Content in Autologous Conditioned Serum

Patients n = 21	*IL-1Ra pg/mL*	*IL-1b pg/mL*	*IL-6 pg/mL*	*IGF-1 ng/mL*	*TGF-b1 ng/mL*
ACS (24 hours at 37°C)	2913.2 ± 451.2	40.4 ± 24.9	58.9 ± 13.9	140.6 ± 41.8	225.6 ± 196.4

ACS was analyzed for cytokine and growth factor content by ELISA. ACS showed elevated concentrations of the measured factors shown. Data given in pg/mL or ng/mL ± SD.

ACS, autologous conditioned serum; ELISA, enzyme-linked immunosorbent assay; IGF, insulin-like growth factor; IL, interleukin; SD, standard deviation.

counting of leukocytes was performed, but routine blood smear microscopy indicated a very low count.

Patients

The study clinic has a high female to male patient ratio; thus, a female-only cohort was included. One hundred and twenty-three eligible knee patients were diagnosed for chronic OA with Kellgren and Lawrence (K&L) grade II–III. They received i.a. knee injections with either ACS (2.5 mL) six times, twice per week, or PRP (5 mL) six times, twice per week. Patients were additionally subdivided into two groups by clinical examination: *group subclinical synovitis* (GSS) and *group moderate synovitis* (GMS). Both groups were treated with either ACS or PRP (Table 2).

Clinical and analytical follow-up

Visual analog scale (VAS) for pain and Western Ontario and McMaster Universities Arthritis Index (WOMAC) were used for clinical evaluation. SF viscosity, cytokines IL-1Ra and IL-1b, nitrate, and CDs were used for analytical evaluation.

Statistical analysis

The number of patients was not chosen by statistical considerations. Analysis was performed on a PC Intel CORE i3 using software, including Excel spreadsheets 10.00, statistical software packages Primer of Biostatistics (Version 4.03. Copyright 1998. McGraw Hill), and STATISTICA® (Data analysis software system; StatSoft, Inc.) release 7.0. Means and standard deviations ($M \pm SD$) were calculated. The reliability of the indicators was determined using Student's *t*-test, based on the assumption that the samples being compared belong to the normal distributions. In the nonparametric distribution of the indices, Wilcoxon, Mann-Whitney, and χ^2 tests were used. To assess the reliability of repeated events, an analysis of variance (F) was carried out. Correlation analysis was carried out using the Spearman's rank correlation coefficient (r). The confidence level was assumed to be $p < 0.05$. Cohen's d effect size d is a measure of the strength of the difference between two variables. Cohen's d was interpreted as small (0.2), medium (0.5), or large (0.8). Sawilowsky extended this interpretation to very large (1.2) and huge (2.0).[26]

Table 2. Baseline Data on Patients Included in This Study

	Knee OA subclinical synovitis		*Knee OA moderate synovitis*	
Treatment	*ACS*	*PRP*	*ACS*	*PRP*
Patients (n)	26	30	39	28
Age (years)	56.6 ± 11.0	57.9 ± 8.3	61.2 ± 8.4	64.2 ± 7.7
BMI (kg/m^2)	32.4 ± 4.6	30.7 ± 4.2	31.2 ± 5.2	33.7 ± 4.9
OA duration (years)	7.4 ± 4.8	7.9 ± 2.3	9.8 ± 5.4	10.3 ± 2.9

Female patients with OA grade II–III (Kellgren and Lawrence) were diagnosed with subclinical or moderate synovitis and then appointed to either ACS or PRP injections according to protocol. Numbers are given ± SD.

BMI, body mass index; OA, osteoarthritis; PRP, platelet-rich plasma.

Results

Safety

No substance-related adverse event effects were reported.

Clinical results

Visual analog scale. At the 1- and 3-month follow-up, VAS pain improved significantly versus baseline in all groups, except for PRP at 3 months in GMS. p-Values and intragroup effects sizes for ACS were significantly superior to PRP at 3 months post-treatment:

GSS: ACS versus PRP effect size d: 1.67 versus 1.13; $p = 0.03$, respectively. GMS: ACS versus PRP d: 1.47 versus 0.28; $p = 0.000$, respectively (Table 3).

WOMACglobal. At the 1- and 3-month follow-up, WOMACglobal improved significantly versus baseline in all groups, except for PRP at 3 months in the GMS. p-Values and intragroup effects sizes for ACS were significantly superior to PRP at 3 months post-treatment.

GSS: ACS versus PRP d: 1.25 versus 0.96; $p = 0.044$, respectively. GMS: ACS versus PRP d: 1.24 versus 0.14; $p = 0.000$, respectively (Table 4).

Analytical results

Concentrations of SF IL-1Ra and IL-1b were determined from aspirates taken at baseline and 1 month after treatment. In both groups, ACS and PRP, IL-1b declined significantly at 1 month after treatment ($p = 0.002$ vs. $p = 0.015$). This decline was significantly stronger with ACS than PRP ($p = 0.008$). In both groups IL-1Ra increased significantly at 1 month ($p = 0.001$ vs. $p = 0.042$). This increase was significantly stronger with ACS than PRP ($p = 0.016$) (Table 5). No SF cytokines were measured at a later follow-up.

SF viscosity. For organizational reasons, the time points of follow-up differed between groups. The ACS group was followed until day 180 and the PRP group was followed until day 90. At all time points, the relative viscosity was significantly higher than at baseline. The ACS group showed a steady incline of viscosity until day 180, the PRP group showed a steep incline at day 8, and thereafter a steady decline until day 90 (Table 6).

SF CD concentration. Significant reductions of CD concentrations were detected with ACS at all follow-up points ($p \leq 0.006$) versus baseline and versus PRP. PRP did not change CD concentrations (Table 7).

SF nitrate concentration. Significant reductions of NO_3 concentrations were detected with ACS at all follow-up points ($p = 0.000$) versus baseline and at 3 months versus PRP. NO_3 in the PRP group significantly dropped at 1 month, but increased again at 3 months (Table 8).

Table 3. Clinical Outcomes by Visual Analog Scale

		VAS (mm) ± SD		
OA knee characteristics	*Treatment*	*Baseline (0)*	*1 month (1)*	*3 months (3)*
Subclinical synovitis	ACS $n=26$	56.4 ± 12.5	38.2 ± 12.2 −32.3% $p=0.000$	30.3 ± 18.2 − 46.3% $p=0.000$ *d*: 1.67
	PRP $n=30$	56.1 ± 15.8	35.4 ± 13.1 −36.8% $p=0.000$	39.6 ± 13.3 −29.4% $p=0.000$ *d*: 1.13
Significance between groups		$p=0.93$	$p=0.41$	$p=0.03$
Moderate synovitis	ACS $n=39$	64.0 ± 12.8	44.0 ± 17.6 −31.3% $p=0.000$	34.0 ± 17.4 −46.9% $p=0.000$ *d*: 1.47
	PRP $n=28$	59.4 ± 17.4	48.3 ± 15.7 −18.6% $p=0.015$	54.7 ± 16.4 −7.9% $p=0.303$ *d*: 0.28
Significance between groups		$p=0.22$	$p=0.31$	$p=0.000$

One hundred and twenty-three patients diagnosed with subclinical and moderate synovitis symptomology were treated by intra-articular injections of 6×2.5 mL ACS or 6×5 mL PRP. Patients received two injections per week. Subjective VAS evaluation was obtained at baseline and after 1 and 3 months. *p*-Values are given versus baseline and as percent changes. Also, *p*-values are given between groups.
VAS, visual analog scale.

Discussion

Intra-articular injections of ACS and PRP have been in use for more than 10 years. So far, a direct comparison between these two modalities was missing.

In this study, both ACS and PRP induced clinical improvements (Tables 3 and 4). One difference was the markedly stronger efficacy of ACS in GMS cases. In addition, ACS efficacy further increased between the 1- and 3-month follow-up as measured in both WOMACglobal and VAS,

Table 4. Clinical Outcomes by Western Ontario and McMaster Universities Arthritis Index

		WOMACglobal ± SD		
OA knee characteristics	*Treatment*	*Baseline*	*1 month*	*3 months*
Subclinical synovitis	ACS $n=26$	57.3 ± 13.4	41.4 ± 10.4 −27.8% $p=0.000$	40.7 ± 13.2 −28.9% $p=0.000$ *d*: 1.25
	PRP $n=30$	63.4 ± 16.2	47.9 ± 13.8 −24.4% $p=0.000$	48.5 ± 14.9 −23.5% $p=0.000$ *d*: 0.96
Significance between groups		$p=0.13$	$p=0.055$	$p=0.044$
Moderate synovitis	ACS $n=39$	64.2 ± 15.0	48.5 ± 12.9 −24.5% $p=0.000$	45.8 ± 14.6 −28.7% $p=0.000$ *d*: 1.24
	PRP $n=28$	65.1 ± 15.7	61.3 ± 14.2 −5.8% $p=0.346$	62.9 ± 15.1 −3.4% $p=0.595$ *d*: 0.14
Significance between groups		$p=0.81$	$p=0.000$	$p=0.000$

One hundred and twenty-three patients diagnosed with subclinical and moderate synovitis symptomology were treated by intra-articular injections of 6×2.5 mL ACS or 6×5 mL PRP. Patients received two injections per week. Subjective WOMAC evaluation was obtained at baseline and after 1 and 3 months. *p*-Values are given versus baseline and as percent changes. Also, *p*-values are given between groups.
WOMAC, Western Ontario and McMaster Universities Arthritis Index.

Table 5. Concentrations of Intra-Articular Cytokines IL-1Ra and IL-1b

	IL-1b pg/mL mean ± SD		*IL-1Ra pg/mL mean ± SD*	
OA knee moderate synovitis	*Baseline*	*1 month*	*Baseline*	*1 month*
ACS $n = 11$	5.58 ± 2.19	3.22 ± 0.24 −42.3% $p = 0.002$	124.9 ± 69.5	972.7 ± 746.3 +678.7% $p = 0.001$
PRP $n = 10$	5.36 ± 2.15	3.53 ± 0.24 −34.1% $p = 0.015$	126.5 ± 65.7	311.6 ± 259.2 +146.3% $p = 0.042$
Significance between groups	$p = 0.819$	$p = 0.008$	$p = 0.957$	$p = 0.016$

When possible, SF samples were obtained from patients treated with ACS or PRP at baseline and 1 month after treatment. IL-1b and IL-1Ra were determined by ELISA. *p*-Values are given versus baseline and as percent changes. Also, *p*-values are given between groups.
SF, synovial fluid.

Table 6. Viscosity of Synovial Fluid Biopsies in Groups Autologous Conditioned Serum and Platelet-Rich Plasma

		Follow-up (day)				
	Baseline	*11*	*18*	*30*	*90*	*180*
OA knee	*Relative viscosity of SF mean ± SD*					
ACS $n = 20$	6.31 ± 1.40	8.59 ± 1.50 +36.1% $p = 0.000$	7.87 ± 1.32 +24.7% $p = 0.000$	8.89 ± 1.70 +40.9% $p = 0.000$	10.89 ± 2.34 +72.6% $p = 0.000$	10.84 ± 1.18 +71.8% $p = 0.000$
		Follow-up (day)				
	Baseline	*8*	*16*	*24*	*30*	*90*
OA knee	*Relative viscosity of SF mean ± SD*					
PRP $n = 16$	6.02 ± 1.53	11.49 ± 3.23 +90.8% $p = 0.000$	10.96 ± 3.18 +82.0% $p = 0.000$	9.49 ± 1.08 +57.6% $p = 0.000$	8.92 ± 0.4 +48.2% $p = 0.000$	7.34 ± 1.87 +21.9% $p = 0.000$

Viscosity of SF was determined in groups ACS and PRP from SF samples obtained at baseline, at time points of injections, and at later time points when possible. *p*-Values are given versus baseline and as percent changes.

Table 7. Conjugated Diene Concentration in Synovial Fluid Biopsies

OA knee moderate synovitis	*Conjugated dienes in SF μmol/L ± SD*		
	Baseline	*1 month*	*3 months*
ACS $n = 22$	2.75 ± 0.99	1.78 ± 0.77 −35.3% $p = 0.000$	1.28 ± 0.72 −53.5% $p = 0.000$
PRP $n = 15$	2.34 ± 0.77	2.60 ± 0.92 +11.1% $p = 0.408$	2.51 ± 0.87 +7.3% $p = 0.575$
Significance between groups	$p = 0.186$	$p = 0.006$	$p = 0.000$

CD concentration in SF was determined from SF samples obtained at baseline, and 1 and 3 months. *p*-Values are given versus baseline and as percent changes. Also, *p*-values are given between groups.
CD, conjugated diene.

Table 8. Nitrate Concentration in Synovial Fluid Biopsies

OA knee moderate synovitis	*NO_3 in SF mmol/L ± SD*		
	Baseline	*1 month*	*3 months*
ACS $n = 22$	2.23 ± 1.04	1.23 ± 0.74 −44.8% $p = 0.000$	1.22 ± 0.78 −45.3% $p = 0.000$
PRP $n = 15$	2.82 ± 1.59	1.36 ± 0.34 −51.7% $p = 0.002$	1.99 ± 0.96 −29.4% $p = 0.095$
Significance between groups	$p = 0.180$	$p = 0.530$	$p = 0.011$

Nitrate concentration in SF was determined from SF samples obtained at baseline, and 1 and 3 months. *p*-Values are given versus baseline and as percent changes. Also, *p*-values are given between groups.

Table 9. Summary of the Findings

	Clinical parameters at endpoints		*Analytical parameters at endpoints*				
Parameter	*VAS*	*WOMAC*	*IL-1b*	*IL-1Ra*	*NO_3*	*CD*	*Viscosity*
Effect through							
ACS	++	++	+	++	++	++	++ (180 days)
PRP	+[a]	+[a]	+	+	+	−	++ (90 days)

Qualitative summary of the clinical results and SF analyses presented in this article, (++) = strong effect; (+) = effect; and (−) = no effect. In summary, ACS is significantly more efficacious compared to PRP.
[a]Effect in subclinical synovitis only.

while PRP efficacy tended to decline at 3 months. At 3 months, the efficacy of ACS was significantly better than of PRP. ACS patients surpassed the Minimal Clinically Important Improvement (MCII) of 40.9%[27] for knee OA pain (VAS), measuring ~46% improvement, while PRP did not. Previous ACS studies reported progressive or static improvement extending to 6 or 12 months.[8,9] In this study, follow-up was terminated at 3 months. Recently, promising ACS treatments of PRP nonresponders were published. Thirty knee OA patients (median age: 66 years, K&L I-IV)) were treated with four i.a. injections of ACS. An ~67% responder rate was seen at 6 months (responder VAS: improvement by ≥20 mm; responder Lequesne index ≥1 change).[28] Results were *largely* independent of patient age and OA stage, as were in Baselga García-Escudero.[9] This study suggested that OA related joint replacement may be postponed.

ACS is a cell-free BCS

The nonrandomized clinical results presented here appear to be in agreement with previous clinical trials with up to 2 years of clinical follow-up.[8,9] ACS is harvested hours after the initial coagulation, when regenerative factors have accumulated. It contains solute serum components, including potential radical scavengers,[29,30] and cannot cause i.a. fibrin formation. In addition, ACS contains solute cytokines and GF generated by the baseline-composition blood. Also included are extracellular vesicles (EV, including Exosomes). Numbers for plasma EVs have been determined at around 10^{10} per mL.[31] ELISA for CD9, an exosome marker, indicated that during ACS processing, EV numbers further increase (data not shown). ACS derived EV have been described as potential ameliorators of (auto)immune pathologies.[32] As a natural resolution mechanism, blood clots also produce specialized proresolving mediators, SPM,[33] which are part of the omega 3 fatty acid metabolome. SPM trigger resolution of inflammation and tissue regeneration.

PRP is a cell preparation

In early-stage OA and younger patients, PRP appears to have potential. In this study, PRP had no significant clinical efficacy in joints with moderate synovitis (GMS).

The joint space has limited access to oxygen and nutrients. Injection of $>10^9$ of PRP-borne cells drastically increases consumption. This adds stress to an already stressed/inflamed environment and may increase the likelihood of adverse reactions, such as flares (up to 1 in 10 patients).[34,35] In addition, platelets exposed to an inflammatory environment may amplify this.

Paradoxical effects of PRP have been described[36–39] and PRP from older, male, OA patients may even depress chondrocyte metabolism and upregulate inflammation *in vitro*.[40] Coagulation is a prerequisite for platelet degranulation. Textor et al. showed for equine joints that GF and cytokine release from i.a. PRP is influenced by co-administrated Ca^{2+} or thrombin.[41] It thus remains unclear if GF are always released quantitatively from injected PRP. Clinically, it is known that blood (PRP?) does not coagulate classically in a joint.[42] One reason is lack of i.a. thrombin. Rather, plasma injected into rabbit joints deposited fibrin in the synovial membrane.[43]

Different PRP preparations display very different biological characteristics and clinical studies, including the present, remain difficult to compare.[44,45] Initiatives toward standardization have been put forward in the interest of study comparability.[46–48] In most studies, side effects of i.a. PRP are reported ''benign,'' ''transient,'' or ''self-limiting'' with unclear specifications. This study did not find any specific adverse effect by neither ACS nor PRP.

Cytokines and SF viscosity

Increased endogenous IL-1Ra production after ACS injection has previously been observed in a clinically successful study in equine metacarpal joints.[49] In the study presented here, PRP increased endogenous IL-1Ra production and decreased IL-1b production, however, significantly less than ACS. The viscosity of SF is a hallmark of a health joint. It is easy to measure and gives an impression of the state of homeostasis. The long-term, steadily growing improvement by ACS sets this treatment apart from PRP, which achieves a strong, but short improvement. TGFb1 is known to be immuno modulatory and a major stimulator of hyaluronan synthesis in joint cells.[50,51] Since ACS contains about 220 ng/mL TGFb1 (Table 1), it is conceivable that this contributes to the viscosity normalization seen with ACS until day 180. However, PRP is also known to release high amounts of TGFb1. It is conceivable that this triggers the initial strong viscosity increase in the PRP group. It is, however, unclear why this effect abates by day 90.

Reactive oxygen species

ROS are important signals, contributing to homeostasis. However, overproduction of ROS in OA is harmful and changes i.a. signaling, chondrocyte survival, and matrix metabolism. ACS—not PRP—strongly reduced CDs, a footprint of ROS, which aggravate age-associated cartilage damage and contribute to synovial inflammation and dysfunction of subchondral bone (involving, e.g., NF-kB

activation). They directly inhibit proteoglycan synthesis in cultured cartilage and can chemically break up aggrecan and hyaluronic acid (HA).[52] HA fragments in turn may activate NO synthase in chondrocytes.[53] One source of ROS are dysfunctional mitochondria, a sign of metabolic/oxidative stress or senescence, correlated to OA.[54–56] Interestingly, ROS appear to have a decisive role in preventing macrophage M1 to M2 polarization[57] and have been described for equine arthritic joints also.[58]

Nitric oxide

Intra-articular NO radicals act proinflammatory, promoting IL-1b and TNFa expression. They exacerbate joint effusion and cartilage destruction, inhibit total protein synthesis and IL-1Ra production *in vitro*,[59,60] and have cytostatic activity. Interestingly, NO-mediated chondrocyte cell death appears to require additional ROS.[61] NO is rapidly transformed into equimolar concentrations of NO_3 and NO_2. A conversion of NO_2 to NO_3 was not performed before measurement in this study. This leaves a possibility that the amount of i.a. NO was higher than the NO_3 reported in this study. Both ACS and, lesser, PRP reduced SF NO_3 concentration. The duration of clinical efficacy observed in previous studies[8,9] might be explicable by a mechanism of improved joint homeostasis, including macrophage behavior. Briefly, M1-type macrophages are antimicrobial and proinflammatory, increasing NO, ROS, IL-1b, and TNFa load. M2-type macrophages are inflammation resolving and regenerating, increasing IL-1Ra, IL-4, IL-10, and SPM release. A role of macrophages in inflammation and OA is well established.[62–64]

Clinical studies for OA therapy should routinely include SF analysis.

OA is a disease of the whole joint as an organ, not only cartilage integrity.[2] As such, the joint is subject to the normal aging process. Peripheral blood biomarkers are unreliable; therefore, routine inclusion of SF analysis in OA therapy studies is advocated. Integrating data from array and omics analyses and systems biology with SF analysis and clinical results may expedite treatment development. Cellular anomalies/death of cells (pyknosis, karyorrhexis, and karyolysis) are commonly seen in OA SF and should also be considered for analysis. In OA, chondrocytes display increased senescence markers, such as senescence-associated beta-galactosidase (beta Gal) activity.[65] Already, clinical studies aim at the elimination of senescent cells to enable rejuvenation of tissues.[66] OA is one target of such developments, both as a model and as a disease. ACS appears to be effective at advanced age and OA stage. Therefore, possible effects on senescent cells in the joint should be evaluated.

Summary

This study found that ACS and PRP were safe and exerted a differential therapeutic effect on knee OA. PRP was not effective in cases of moderate synovitis, ACS was. ACS was significantly superior to PRP in all groups at the 3-month follow-up and met the MCII criteria for VAS in knee OA. Table 9 qualitatively summarizes the clinical and analytical findings of this study.

PRP and ACS injections had differential effects on SF markers of inflammation: cytokine dysbalance, viscosity, and NO_3. CD concentration (no effect by PRP) and overall biochemical efficacy of ACS were significantly superior to PRP. Published ACS studies consistently confirm its potential for regenerative action.

The authors conclude that ACS, superior to PRP, improved joint homeostasis in this female knee OA patient cohort. Analytical data displayed possible rejuvenation-associated characteristics in the sense that ROS footprint CDs were reduced. ROS by itself can damage DNA and further drive ROS production and senescence.[67] Joint macrophages as part of the innate immune system might possibly play a role. Published ACS studies consistently confirm its potential for regenerative action.

Clinical studies intergrating meaningful aspects of joint organ homeostasis with clinical scores are advocated.

This study lacked a double-blinded protocol. The small number of patients per group and the lack of male patients are reasons for caution. Detailed identification of SF cells was not performed and CDs and NO_3 are surrogate parameters for their causative agents (ROS and NO). The presence of endogenous radical scavengers such as glutathione, uric acid or bilirubin was not analyzed.

Parts of the data have been presented in an abstract at the 2017 OARSI World Congress on Osteoarthritis: Promoting Clinical and Basic Research in Osteoarthritis,[68] Las Vegas, NV.

Author Disclosure Statement

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Address correspondence to:
Julio Reinecke
ORTHOGEN AG
Ernst Schneider Platz 1
Düsseldorf 40212
Germany

E-mail: julio.reinecke@orthogen.com

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