Systemic effects after local injection of platelet-rich plasma: a prospective randomized study

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Abstract

Background Platelet-rich plasma (PRP) is widely utilized in the treatment of sports injuries. However, potential systemic effects after localized PRP injection are unclear at present.

Design: prospective randomized study

Methods Twenty-four Taiwanese male athletes with tendinopathy were randomized into a PRP group (n = 13) or a saline group (n = 11).

Results The results showed no significant differences in serum levels of growth hormone, insulin-like growth factor-1, insulin-like growth factor-binding protein 3, vascular endothelial growth factor, platelet-derived growth factor-BB, or serum substance P between the two groups at baseline, nor at 1, 2, or 7 days after intervention. However, a significant decrease in the serum substance P level 1 and 7 days after PRP injection was observed. Regarding urinary concentrations of metabolites of anabolic androgenic steroids (AAS), no between-group differences at baseline, nor at 1, 2, or 7 days after intervention, were observed.

Conclusions Our study failed to observe significant surge of serum anabolic molecules and urinary excretion of anabolic AAS metabolites after PRP injection.

Trial registration with ClinicalTrials.gov: NCT04456907

Background

Tendinopathy is a significant problem in sport and can interfere with and, in some instances, end an athletic career(1). As a result, the treatment of tendinopathy is of pivotal importance for the health and career of the athletes. Platelet-rich plasma (PRP) is widely-utilized in the treatment of sports injuries, including tendinopathy(2). The administration of PRP can change the tissue microenvironment by providing a pool of regenerative molecules, thereby enhancing angiogenesis and activating the chemotaxis and proliferation of the regenerative cells(3). These beneficial anabolic molecules include, but are not limited to, growth hormone (GH), insulin-like growth factor-1 (IGF-1), insulin growth factor-BP3 (IGF-BP3), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), granulocyte-colony stimulating factor (G-CSF), hepatocyte growth factor (HGF), and transforming growth factor- beta 1 (TGF- β 1)(4). TGF- β 1 has been known to exert anabolic effects on cartilage and anti-inflammatory effects on synovial tissues(5).

In the sporting competitions, the term “doping” is defined as the use of prohibited athletic enhancing agents by the competitive athletes. Blood and urine samples are frequently utilized for the doping tests. Due to concerns surrounding unfair enhancement of athletic performance in competitive sports, the use of PRP among athletes is under dispute. Platelet-derived preparations were included on the 2010 World Anti-Doping Agency (WADA) prohibited list, but were later removed in 2011 due to a lack of strong evidence regarding performance benefits. In addition to the controversy surrounding the labeling of PRP

use as doping, the systemic effect of localized PRP injection is far from clear at present. No prospective randomized study has demonstrated systemic effects after localized PRP injection, and there is no evidence to support or refute whether the use of PRP could lead to misinterpretation of blood or urinary doping tests.

The purpose of the research was to quantify the impact of local PRP injection on the serum concentrations of anabolic molecules and on the urinary excretion of anabolic androgenic steroids (AAS) metabolites, the frequently measured markers in the urinary doping tests. This prospective randomized study tried to determine PRP injection could exert different effects on the expression of serum concentrations of anabolic molecules and on the urinary excretion of anabolic AAS metabolites than the placebo saline injection, in order to determine whether PRP injection is a concern for the blood and urinary doping tests.

Methods

This double-blinded, placebo-controlled trial was performed from Jan 1st, 2017 to Dec 31st, 2017 in accordance with CONSORT guideline. Ethical approval was granted by the Institutional Review Board of the Chang Gung Memorial Hospital (CGMH-IRB No: 201700133A3). Patients were recruited from a sports medicine clinic held in the Department of Orthopedic Surgery, Kaohsiung Chang Gung Memorial Hospital. All of the authors identify the committee that approved the research and confirm that all research was performed in accordance with relevant guidelines/regulations, and the details of the study were reported referencing upon the Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO) guideline proposed by Murray IR et al(6).

Informed consent was obtained and that the rights of participants were protected for all of the participants.

Recruitment of participants

Male Asian athletes between 18 and 40 years of age with upper or lower limb tendinopathy for at least 3 months were enrolled in the study with the details of tendinopathy summarized in Table 1. The diagnosis of tendinopathy was confirmed through the provocation test, ultrasonography or MRI by at least two of three orthopedic surgeons (J.Y.K, C.C.H and W.Y.C).

Table 1
Demographic data of the participants in the PRP group and saline groups.

	PRP Group	Saline Group	p value
Gender	13 men	9 men	
Age (yrs)	29.0 (23.0,33.0)	24.0 (21.0,25.0)	0.278
BMI	24.5 (22.0,27.5)	22.6 (20.5,23.2)	0.243
Clinical diagnosis			
Rotator cuff tendinopathy	2	1	
Lateral epicondylitis	4	3	
Medial elbow tendinopathy	3	2	
Gluteal Tendinopathy	1	0	
Patellar tendinopathy	1	1	
Achilles tendinopathy	1	2	
Peroneal tendinopathy	1	0	
Maximal heart rate (beats/min)	192 (183, 200)	195 (186, 200)	0.278
Exercise frequency (per week)	4 (2, 7)	4 (2, 6)	0.263
Medical diseases	N/A	N/A	
BMI: body mass index			

The exclusion criteria were as follows: (1) nutritional disorders, (2) hematologic or systemic diseases (ex: anemia, metabolic disease...etc.), (3) history of hormone therapy, (4) use of anti-inflammatory agents, anti-platelet agents, or traditional Chinese herbs within the past month, (5) surgical history for current injury, and (6) biologic treatment for current injury [14]. Patients were required to be available for all scheduled appointments during the follow-up period.

The minimum sample size required for each group was calculated before the study. The priori power calculation (G*Power 3.1.9.2 software: <http://www.gpower.hhu.de/en.html>) utilized a 1-tailed Wilcoxon signed-rank test to calculate the sample size of at least 9 for each group (calculated effect size: 1.2; α level: 0.05; power: 80%; allocation ratio: 1)(7).

Process of randomization

The unblinded independent research assistant (Y.T.Z) randomized the eligible participants into the PRP group or the saline group using suitable computer software. The participants randomized into the PRP

group were treated with 4 ml PRP, while the participants in the saline group received a saline injection of equal volume. On the day of intervention, each subject donated a 20-ml blood sample and a 50-ml urine sample after intervention. After 1 hour of preparation, the research assistant selected the correct syringe and blinded the content with the use of a covering sheath to surround the syringe and hub of the needle. To ensure concealment of the subject's group allocation, data on allocation were stored in a secret location. The content of the injection was blinded for the orthopedic physicians, researchers, and patients. Treatment-related complications and assessment of pain intensity using the Numeric Rating Scale (NRS) were recorded by the independent research assistant.

Preparation of platelet-rich plasma (PRP)

Autologous platelet-rich plasma (PRP) was prepared using the RegenKit THT system (RegenLab SA, Le Mont-sur-Lausanne, Switzerland) following the manufacturer's instructions. Medical technicians, who had been well-trained by the manufacturer, were responsible for the process of PRP preparation. For each patient, 8–10 mL of venous blood was drawn and collected to the commercial RegenLab THT tube, which contained 1 mL sodium citrate. After single centrifugation at 3400 revolutions per minute (rpm) for 8 minutes, 4–5 mL of PRP was yielded with leukocytes maintained at physiological levels and red blood cells depleted. Then, the blood components were separated, with the platelet pellet resting on the separating gel. PRP for later application was obtained by re-suspending the platelet pellet in the plasma supernatant by gently inverting the unopened RegenKit THT tube 5 to 10 times. Finally, we collect the supernatant fraction using the syringe equipped with a 5-ml Luer Lok syringe without any activating agent. All the steps were performed in room air and were completed within 60 minutes before the injection. The whole blood characters were counted in Kaohsiung Chang Gung memorial hospital.

The three orthopedic surgeons (J.Y.K, W.Y.C and C.C.H) delivered 4 mL PRP or saline to the respective intratendinous lesions. Patients were advised not to take anti-inflammatory medications during the first 7 days after the injection. Physical therapy or heavy training was forbidden for 7 days after the intervention, and the daily activities were allowed. Individualized rehabilitation was instructed and supervised by a physical therapist 7 days after the intervention.

Quantification of the concentrations of serum biomarkers

A blood sample of approximately 20 mL was taken from the brachial vein of the untreated limb 1 hour before and at 1, 2, and 7 days after PRP injection. To mitigate the confounding effects of diurnal variation and the metabolic effects of diet and acute bouts of exercise, blood was drawn at precisely the same time each morning between 8 and 10 AM and at least 6 hours after eating or training [12,18].

The blood specimen was centrifuged at $3000 \times g$ for 10 minutes and then stored at -80°C until Enzyme-Linked Immunosorbent Assay (ELISA) assessment. The concentrations of target proteins were assessed using a Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions. Results were calculated by interpolation from a standard curve established from graded concentrations of GH (DGH00, R&D Systems), IGF-1 (DG100, R&D Systems), IGFBP-3 (DGB300, R&D

Systems), PDGF-BB (DBB00, R&D Systems), VEGF (DVE00, R&D Systems) and SP (KGE007, R&D Systems).

Quantification of doping substances in urine

Doping substances in urine, mainly metabolites of anabolic androgenic steroids (AAS), were quantified in the laboratory of the Super-Micro Mass Research and Technology Center, Cheng Shiu University, Taiwan. The metabolites of AAS included testosterone (17 β -hydroxyandrost-4-en-3-one), epitestosterone (17 α -hydroxy-4-androsten-3-one), androsterone (4-androsten-3,17-dione), etiocholanolone (3 α -hydroxy-5 β -androstan-17-one), DHEA (dehydroepiandrosterone), dihydroandrosterone (5 α -androstan-3 α ,17 β -diol), and etiocholane-3 α ,17 β -diol (5 β -androstan-3 α ,17 β -diol). Each urine sample (6 mL) was mixed with 50 μ L standard solution (5000 ng/mL methyltestosterone and androsterone-D4 6000 ng/mL) and 1 mL of phosphate buffer, and the mixture was heated for 60 min at 50 °C. After cooling at room temperature, liquid–liquid extraction was performed, and phase separation was achieved. The organic extract was evaporated to dryness, and the dried residue was further derivatized with 50 μ L of N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) solution for 30 min at 60 °C. Finally, the sample was subjected to gas chromatographic analysis (7890A Network GC system) and mass spectrometric analysis (5975C Network Mass Selective Detector) for quantification of doping substances of interest.

Statistical Analysis

All data were expressed as the median (lower quartile, upper quartile). Categorical variables were compared using the Chi-square test. The Mann-Whitney U test was utilized to compare inter-group differences. The Friedman test was utilized for repeated-measures analysis of repeated within-group comparisons for continuous variables, and the Wilcoxon signed-rank test was used for *post hoc* analysis. The corrections for multi-comparisons were performed for all pertinent tests.

Statistical analysis was performed using PASW Statistics Statistical software (version 10.0; SPSS, Chicago, IL, USA). A result was considered statistically significant at $p < 0.05$ (7).

Results

Thirty male Taiwanese athletes fulfilling the inclusion criteria were evaluated in terms of their eligibility for enrollment in this study, and 6 were excluded due to recent use of anti-inflammatory agents, abnormal biochemical studies or recent injuries. The remaining 24 participants were randomized into the PRP group ($n = 13$) or the saline group ($n = 11$). Two participants in the saline group failed to complete all the surveys, and so the results of the remaining 22 participants were included for final analysis. The flowchart of our study is summarized in FIGURE 1. The gender, age, body mass index, maximal heart rate, training frequency, and chronic diseases were similar in the two groups (Table 1).

The serum levels of selected biomarkers, including GH, IGF-1, IGFBP-3, VEGF, PDGF-BB, and SP, showed no between-group differences at baseline, nor at 1, 2, or 7 days after intervention (Table 2).

Table 2
Comparison of serum biomarkers between the PRP and saline groups.

GH (pg/mL)	PRP Group	Saline Group	*p value
Baseline	33.6 (29.1,58.9)	71.0 (40.6,183.8)	0.433
Day 1	49.3 (29.9,73.0)	176.7 (67.4,420.3)	0.836
Day 2	47.4 (28.2,397.8)	126.2 (39.1,334.0)	0.855
Day 7	57.5 (43.8,221.9)	114.3 (88.4,173.5)	0.862
IGF-1 (ng/mL)	PRP Group	Saline Group	*p value
Baseline	85.8 (72.7,99.1)	84.7 (76.4,99.8)	0.835
Day 1	81.0 (74.9,110.0)	100.2 (74.5,115.9)	0.899
Day 2	88.7 (77.2,102.2)	91.9 (75.3,98.6)	0.718
Day 7	90.5 (83.9,104.9)	90.1 (71.5,110.9)	0.709
IGFBP-3 (ng/mL)	PRP Group	Saline Group	*p value
Baseline	2859.5 (2690.4,3212.7)	2325.7 (2319.6,2896.3)	0.225
Day 1	3038.6 (2806.8,3293.9)	3013.3 (2313.0,3325.0)	0.630
Day 2	2991.5 (2623.5,3429.6)	2932.0 (2527.3,3169.4)	0.475
Day 7	3002.2 (2786.3,3363.2)	2850.7 (2770.3,2923.1)	0.133
VEGF (pg/mL)	PRP Group	Saline Group	*p value
Baseline	252.5 (141.5,346.1)	242.6 (124.5,265.5)	0.198
Day 1	278.0 (203.3,339.6)	247.9 (133.9,307.2)	0.195
Day 2	239.9 (171.2,288.0)	172.2 (117.8,308.3)	0.268
Day 7	249.0 (172.2,288.0)	231.0 (145.6,261.9)	0.421
PDGF-BB (ng/mL)	PRP Group	Saline Group	*p value
Baseline	3559.7 (3267.1,5183.8)	3178.3 (2407.8,3631.9)	0.104
Day 1	3268.8 (3062.5,3675.2)	3198.8 (2167.7,3654.1)	0.171
Day 2	3619.1 (3001.7,3877.7)	2471.0 (1792.7,3420.9)	0.072

GH: growth hormone; IGF-1: insulin-like growth factor-1; IGF-BP3: insulin-like growth factor-binding protein 3; VEGF: vascular endothelial growth factor; PDGF-BB: platelet-derived growth factor-BB; SP: substance P

*p value: p value of the Mann-Whitney U test

GH (pg/mL)	PRP Group	Saline Group	*p value
Day 7	3453.4 (3139.4,4788.5)	3056.1 (2623.9,3841.2)	0.186
SP (pg/mL)	PRP Group	Saline Group	*p value
Baseline	236.6 (145.2,274.0)	178.5 (124.1,223.3)	0.228
Day 1	153.0 (137.5,174.0)	128.4 (104.3,179.6)	0.515
Day 2	218.1 (149.1,251.9)	176.2 (139.4,186.4)	0.591
Day 7	124.0 (109.0,176.2)	108.2 (64.6,156.7)	0.851
GH: growth hormone; IGF-1: insulin-like growth factor-1; IGF-BP3: insulin-like growth factor-binding protein 3; VEGF: vascular endothelial growth factor; PDGF-BB: platelet-derived growth factor-BB; SP: substance P			
*p value: p value of the Mann-Whitney U test			

The p values of the Friedman test for GH, IGF-1, IGFBP-3, VEGF, PDGF-BB, and SP in the PRP group were 0.819, 0.764, 0.403, 0.059, 0.231, and < 0.001, respectively (Table 3). The Wilcoxon signed-rank test was used for *post hoc* analysis of the serum level of SP in the PRP group, and the p values for baseline–Day 1, baseline–Day 2, baseline–Day 7, Day 1–Day 2, Day 1–Day 7, and Day 2–Day 7 were 0.016, 0.423, < 0.001, 0.027, 0.034, and < 0.001, respectively (Table 4). These results suggested that injection of PRP may lead to significant changes in the serum level of SP.

Table 3
Comparison of serum biomarkers with baseline values after PRP injection.

GH (pg/mL)	PRP Group	#p value
Baseline	33.6 (29.1,58.9)	
Day 1	49.3 (29.9,73.0)	
Day 2	47.4 (28.2,397.8)	
Day 7	57.5 (43.8,221.9)	0.819
IGF-1 (ng/mL)	PRP Group	#p value
Baseline	85.8 (72.7,99.1)	
Day 1	81.0 (74.9,110.0)	
Day 2	88.7 (77.2,102.2)	
Day 7	90.5 (83.9,104.9)	0.764
IGFBP-3 (ng/mL)	PRP Group	#p value
Baseline	2859.5 (2690.4,3212.7)	
Day 1	3038.6 (2806.8,3293.9)	
Day 2	2991.5 (2623.5,3429.6)	
Day 7	3002.2 (2786.3,3363.2)	0.403
VEGF (pg/mL)	PRP Group	#p value
Baseline	252.5 (141.5,346.1)	
Day 1	278.0 (203.3,339.6)	
Day 2	239.9 (171.2,288.0)	
Day 7	249.0 (172.2,288.0)	0.059
PDGF-BB (ng/mL)	PRP Group	#p value
Baseline	3559.7 (3267.1,5183.8)	
Day 1	3268.8 (3062.5,3675.2)	
Day 2	3619.1 (3001.7,3877.7)	
GH: growth hormone; IGF-1: insulin-like growth factor-1; IGF-BP3: insulin-like growth factor-binding protein 3; VEGF: vascular endothelial growth factor; PDGF-BB: platelet-derived growth factor-BB; SP: substance P		
#p value: p value of the Friedman test		

GH (pg/mL)	PRP Group	#p value
Day 7	3453.4 (3139.4,4788.5)	0.231
SP (pg/mL)	PRP Group	#p value
Baseline	236.6 (145.2,274.0)	
Day 1	153.0 (137.5,174.0)	
Day 2	218.1 (149.1,251.9)	
Day 7	124.0 (109.0,176.2)	< 0.001
GH: growth hormone; IGF-1: insulin-like growth factor-1; IGF-BP3: insulin-like growth factor-binding protein 3; VEGF: vascular endothelial growth factor; PDGF-BB: platelet-derived growth factor-BB; SP: substance P		
#p value: p value of the Friedman test		

Table 4
The p value for the post-hoc analysis of serum level of substance P for the PRP group by Wilcoxon signed-rank test.

	Baseline	Day 1	Day 2	Day 7
Baseline		0.016	0.423	< 0.001
Day 1	0.016		0.027	0.034
Day 2	0.423	0.027		< 0.001
Day 7	< 0.001	0.034	< 0.001	

With regards to the urine concentrations of selected AAS metabolites, including testosterone, epitestosterone, androsterone, etiocholanolone, DHEA, dihydroandrosterone, and etiocholane-3 α ,17 β -diol, no between-group differences were observed at baseline, nor at 1, 2, or 7 days after intervention (Table 5). The p values of the Friedman test for testosterone, epitestosterone, androsterone, etiocholanolone, DHEA, dihydroandrosterone, and etiocholane-3 α ,17 β -diol were 0.873, 0.742, 0.123, 0.270, 0.819, 0.753, and 0.896, respectively (Table 6). These results indicated that PRP injection did not lead to significant changes in the urinary excretion of AAS metabolites.

Table 5
Comparison of urine AAS metabolites between PRP and saline group at each time point.

Testosterone (ng/mL)	PRP Group	Saline Group	*p value
Baseline	4.65 (2.86,11.36)	2.44 (0.93,3.75)	0.219
Day 1	4.72 (2.07,20.59)	3.99 (3.19,5.06)	0.598
Day 2	5.69 (2.23,10.32)	3.56 (2.64,4.06)	0.712
Day 7	4.95 (2.62,17.25)	3.02 (2.22,6.85)	0.631
Epitestosterone (ng/mL)	PRP Group	Saline Group	*p value
Baseline	26.8 (19.7,44.2)	13.1 (11.4,51.9)	0.689
Day 1	19.3 (15.4,56.1)	19.8 (13.2,74.6)	0.827
Day 2	20.6 (15.0,42.5)	31.9 (16.2,62.0)	0.972
Day 7	38.6 (18.5,46.8)	26.4 (19.4,65.5)	0.982
Androsterone (ng/mL)	PRP Group	Saline Group	*p value
Baseline	2281.2 (1823.0,3772.8)	1459.1 (910.6,2708.6)	0.336
Day 1	2122.1 (1250.1,2761.4)	3053.8 (1028.0,4270.2)	0.375
Day 2	2413.8 (1257.4,2675.2)	1534.1 (1138.3,2459.8)	0.946
Day 7	2814.4 (1718.8,3889.2)	2541.6 (982.8,4840.4)	0.681
Etiocholanolone (ng/mL)	PRP Group	Saline Group	*p value
Baseline	1632.4 (1337.4,3187.4)	735.2 (453.1,1741.8)	0.582
Day 1	1303.4 (633.0,1981.8)	1823.4 (1090.9,2011.8)	0.586
Day 2	1104.1 (689.4,2010.6)	1021.5 (817.5,1420.8)	0.985
Day 7	1595.2 (981.7,2204.8)	1443.6 (684.0,2148.4)	0.665
DHEA (ng/mL)	PRP Group	Saline Group	*p value
Baseline	12.91 (9.14,24.9)	3.89 (3.29,11.77)	0.843
Day 1	7.71 (5.47,14.23)	7.61 (3.44,47.69)	0.360
Day 2	13.68 (8.37,15.30)	23.11 (16.22,48.56)	0.098
Day 7	13.00 (6.87,37.78)	17.99 (7.59,63.66)	0.635
Dihydroandrosterone (ng/mL)	PRP Group	Saline Group	*p value
Baseline	69.2 (50.4,81.0)	49.1 (27.4,54.2)	0.074
*p value: p value of the Mann-Whitney U test			

Testosterone (ng/mL)	PRP Group	Saline Group	*p value
Day 1	59.1 (42.1,65.3)	68.6 (17.5,94.3)	0.945
Day 2	54.8 (22.0,97.7)	36.1 (20.6,57.1)	0.257
Day 7	66.5 (40.0,91.1)	71.1 (17.5,92.0)	0.839
Etiocholane-3 α ,17 β -diol(ng/mL)	PRP Group	Saline Group	*p value
Baseline	52.7 (42.1,82.9)	27.9 (13.0,49.5)	0.152
Day 1	62.7 (28.5,115.6)	50.2 (36.6,67.8)	0.347
Day 2	48.8 (20.4,67.0)	22.2 (18.5,77.9)	0.312
Day 7	44.4 (29.0,100.4)	40.5 (18.7,106.5)	0.637
*p value: p value of the Mann-Whitney U test			

Table 6
Comparison of urine AAS metabolite with baseline values after PRP injection.

Testosterone (ng/mL)	PRP Group	#p value
Baseline	4.57 (2.89,9.74)	
Day 1	4.72 (2.07,20.59)	
Day 2	5.69 (2.23,10.32)	
Day 7	4.92 (2.57,6.54)	0.873
Epitestosterone (ng/mL)	PRP Group	#p value
Baseline	26.8 (19.7,44.2)	
Day 1	19.3 (15.4,56.1)	
Day 2	20.6 (15.0,42.5)	
Day 7	38.6 (18.5,46.8)	0.742
Androsterone (ng/mL)	PRP Group	#p value
Baseline	2281.2 (1823.0,3772.8)	
Day 1	2122.1 (1250.1,2761.4)	
Day 2	2413.8 (1257.4,2675.2)	
Day 7	2814.4 (1718.8,3889.2)	0.123
Etiocholanolone (ng/mL)	PRP Group	#p value
Baseline	1632.4 (1337.4,3187.4)	
Day 1	1303.4 (633.0,1981.8)	
Day 2	1104.1 (689.4,2010.6)	
Day 7	1595.2 (981.7,2204.8)	0.270
DHEA (ng/mL)	PRP Group	#p value
Baseline	12.91 (9.14,24.9)	
Day 1	7.71 (5.47,14.23)	
Day 2	13.68 (8.37,15.30)	
Day 7	13.00 (6.87,37.78)	0.819
Dihydroandrosterone (ng/mL)	PRP Group	#p value
*p value: p value of the Mann-Whitney U test		

Testosterone (ng/mL)	PRP Group	#p value
Baseline	69.2 (50.4,81.0)	
Day 1	59.1 (42.1,65.3)	
Day 2	54.8 (22.0,97.7)	
Day 7	66.5 (40.0,91.1)	0.753
Etiocholane-3 α ,17 β -diol(ng/mL)	PRP Group	#p value
Baseline	52.7 (42.1,82.9)	
Day 1	62.7 (28.5,115.6)	
Day 2	48.8 (20.4,67.0)	
Day 7	44.4 (29.0,100.4)	0.896
*p value: p value of the Mann-Whitney U test		

Regarding clinical parameters, no significant between-group difference in NRS was observed between the PRP and saline group. There were no adverse responses associated with the injection in the two groups (Table 7).

Table 7
Comparison of numerical rating scale (NRS) between PRP and saline groups.

	PRP Group	Saline Group	*p value
Baseline	5.0 (3.0,6.0)	4.0 (4.0,5.0)	0.208
Day 1	3.0 (2.0,5.0)	2.0 (1.0,4.0)	0.158
Day 2	2.0 (2.0,4.0)	2.0 (1.0,4.0)	0.163
Day 7	2.0 (1.0,3.0)	3.0 (1.0,5.0)	0.506
*p value: p value of the Mann-Whitney U test			

Discussion

In this study, no significant differences in the serum levels of GH, IGF-1, IGFBP-3, VEGF, PDGF-BB, or SP were observed between the PRP group and saline group at baseline, nor at 1, 2, or 7 days after intervention. However, significant decreases in the serum SP level were observed 1 and 7 days after PRP

injection. Regarding urinary AAS metabolites, including testosterone, epitestosterone, androsterone, etiocholanolone, DHEA, dihydroandrosterone, and etiocholan-3 α ,17 β -diol, no between-group differences at baseline, nor at 1, 2, or 7 days after intervention, were observed. These results have not been reported before and merit note.

PRP has been utilized in the treatment of musculoskeletal diseases, such as osteoarthritis, with favorable functional outcomes of the injected site, despite the fact that the pertinent studies were not free from bias(8). However, the systemic effects after PRP injection are inconclusive at present. Banfi et al. observed significant modifications of serum VEGF, EGF and CCL2 levels 30 minutes after PRP injection, which returned to baseline within 24 hours(9). Wasterlain et al. centrifuged 30 ~ 60 mL whole blood to yield 3 ~ 6 mL PRP. Serum levels increased significantly for IGF-1 at 24 and 48 hours, bFGF at 72 and 96 hours, and VEGF at 3, 24, 48, 72, and 96 hours after PRP injection. Additionally, VEGF was increased in all 25 patients after PRP treatment(10). These studies were all longitudinal observational studies without control groups. In our study, we observed no between-group differences in the serum levels of all selected biomarkers nor in the urinary excretion of AAS metabolites. We did not observe increases in the serum levels of IGF-1, bFGF, or VEGF after PRP injection. The discrepancies between the results of our study and previous studies may be due to differences in the concentration ratio during PRP preparation, the volume of injected PRP, the vascularity of the injection sites, and the differences in the demographic compositions.

We did observe a substantial decrease in the serum SP level after local PRP injection. However, between-group differences could not be demonstrated at baseline, nor at 1, 2, or 7 days after intervention. A decrease in the serum level of SP has not been reported previously. Previous study has suggested a positive correlation between the systemic SP level and pain nociception(11). Lisowska et al. found that among patients with rheumatoid arthritis, the extent of pain after total knee arthroplasty was positively correlated with the serum level of SP (11). The decrease in the serum SP level after PRP injection could be partially explained by decreased nociception due to the healing process after PRP injection. Reviewing the literature, there is no evidence of a correlation between the serum SP level and athletic performance. As a result, the decreased serum SP level after PRP injection does not constitute evidence in support of labelling local PRP injection as doping.

There are limitations to our study. The heterogeneity of the disease composition might jeopardize the comparability of the two groups. The small volume of yielded PRP by the RegenKit THT system did not allow for the component characterization for every participant. The relatively small sample size might make the study underpowered. The findings of our study need to be supplemented by the performance data in the following studies.

Conclusions

Our study failed to observe significant surge of serum anabolic molecules and urinary excretion of anabolic AAS metabolites after PRP injection. Significant decrease in the serum substance P level could

be observed 1 and 7 days after PRP injection.

List Of Abbreviations

bFGF: basic fibroblast growth factor

EGF: epidermal growth factor

G-CSF: granulocyte-colony stimulating factor

GH: growth hormone

HGF: hepatocyte growth factor

IGF-1: insulin-like growth factor-1

IGF-BP3: insulin growth factor-BP3

PDGF: platelet-derived growth factor

PRP: platelet rich plasma

TGF- β 1: transforming growth factor-beta 1

VEGF: vascular endothelial growth factor

Declarations

Ethics approval and consent to participate:

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Consent for publication:

not applicable

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

SJ and CC wrote the manuscript. WY, KK, and JY recruited the patients. GP, SF, and TC performed the laboratory experiments.

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Figures

Figure 1

The flow diagram of the study. The processed of recruitment, randomization, and intervention of the participants were summarized in this diagram.