The molecular systemic and local effects of intratendinous injection of Platelet Rich Plasma in tendinosis: preliminary results on a rat model with ELISA method

Benjamin Dallaudiere[1,2]
Liliane Louedec[2]
Marie Paule Jacob Lenet[2]
Lionel Pesquer[2]
Elvind Blaise[2]
Anne Perozziello[2]
Jean Baptiste Michel[2]
Maryse Moinard[2]
Philippe Meyer[2]
Jean Michel Serfaty[2]

[1] Centre d'Imagerie Ostéo-articulaire, Clinique du Sport de Bordeaux-Mérignac, France
[2] Department of MSK Radiology Department, CHU Pellegrin, Bordeaux, France

Corresponding author:
Benjamin Dallaudiere
Department of MSK Radiology Department, CHU Pellegrin
Place Amélie Léon Rabat
33000 Bordeaux, France
E-mail: benjamin.dallaudiere@gmail.com

Summary

Purpose: **the aim of our study was thus to quantify the effect of Platelet Rich Plasma (PRP) injection on systemic and local growth factors and to identify molecular markers in a rat model of patellar and Achilles tendinosis treated with PRP.**
Material and method**: twenty two rats were used for the study. Two healthy rats were used as control (T-). We induced tendinosis (T+) in 20 rats (80 tendons by injecting under ultrasonography (US) guidance Collagenase 1® (day 0 = D0, patellar=40 and Achilles=40).**
At D3, these 20 rats with tendinosis were separated in treatment by either PRP (PRPT+, n=28), physiological serum (PST+, n=28, control) US-guided intratendinous injection, or without no PRP or PS (T+, n=24, control of natural evolution of tendinopathy). Follow-up at D7, D13, D18 and D25 using serum sample and local tendon removal with ELISA technics and comparison between the 3 groups were performed.
Results: **during biological follow up, comparison of all serum samples of PRPT+, PST+ and T+ groups showed no significant modification of their biological markers at D7, D13, D18 and D25 (p>0.22). Comparison of immunological sample tendon markers of PRPT+, PST+ and T+ groups also showed no significant modification of markers at D7, D13, D18 and D25 (p>0.16) considering each biological marker and also all subgroups confounded.**
Conclusion: **our study strongly suggests that a single intratendinous US-guided injection of PRP in Achilles and patellar T+ doesn't increase biological markers such as growth factors compared to a control group in mid-term and long-term follow-up.**

KEY WORDS: tendinosis, rat, platelet, PRP, ELISA.

Key Points: our goal was to assess the systemic and local molecular effect of intratendinous injection of PRP in tendinosis.
We used patellar and Achilles tendinosis in a rat model with adequate controls.
We precisely defined platelet, leukocyte concentrations in PRP, on a large cohort.
We evaluated PRP biological molecular systemic and local effects.
We provide strong evidence that PRP didn't increase biological markers, particularly growth factors, in serum and tendon dosages in PRP treated tendinosis compared to placebo group in mid-term and long-term follow-up.

Introduction

Tendinosis (T+) is a very common and disabling condition, resulting in impairment of quality of life. Indeed, T+ of the rotator cuff is the most common musculoskeletal cause of shoulder pain in the general population, mainly women between 40 and 65 year old in Europe whereas Achille's T+ affects 5-6% of the general population, especially young men in North America. In most cases, this condition progresses to a disabling pain or tendon rupture[1,2].
The healthy tendon is composed of type 1 collagen and a few elastic fibers, within a ground substance containing cells (tenocytes and tenoblasts) and water. In case of T+, histology mainly shows thinned and disorganized collagen fibers and increased interfibrillar glycosaminoglycans deposition with production of prostaglandins [PGE2, Interleukines (IL6, IL1B), cyclooxygenase (COX2) and matrix metalloproteinase

(MMP1, MMP3) expression]. Neo-angiogenesis and nerve fiber development have also been reported at the beginning of T+ and throughout tendon healing. Inflammatory lesions are rare, but may be associated with tendon rupture[3]. Early treatment of T+ should therefore be recommended to avoid complications.

Several lines of research have been explored for the treatment of T+ and tendon rupture, including Ultrasound (US)-guided fenestration or tenotomy[4], and intratendinous injections of hyperosmolar solutions[5], bone morphogenic protein[6], or platelet-rich plasma (PRP), with varying efficiencies[7,8]. Despite these potential treatments, peri-tendinous injection of corticosteroid remains the commonly accepted strategy to treat diseases of the tendon, despite the absence of inflammation in T+ in this condition, and the proven serious side effects (tendon rupture)[9] due in part to intra-tendinous injection. PRP is defined as plasma with a platelet concentration (from 1,000000 to 2,400000 par μL) 3 to 8 times higher than in blood, which promotes stem cell recruitment and directly stimulates collagen production by the tendon tenoblasts[10] with proliferation and differentiation of human tenocytes in response to PRP. PRP can be directly injected into tendons to enhance local platelet concentration. Numerous *in vitro*[11-13] and animal studies using this technique have been performed in animal models of tendon rupture or T+ with results demonstrating improvement of clinical and histological repair[14,15]. Similarly, human studies have shown discordant results regarding pain reduction in different tendon locations[16].

To our knowledge, no studies have been performed on an animal model to quantify the effect of PRP injection on systemic and local molecular marker as growth factors to permit tendon healing.

Recently, a descriptive laboratory study suggested, in a small number of patients (n=25), that PRP intratendinous injection may trigger systemic increases basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and platelet-derived growth factor-BB (PDGF-BB) in competitive athletes with evidence that VEGF could serve as a useful molecular marker to detect athletes treated with PRP. Unfortunately, the platelet or leukocyte counts in the PRP treatment were not reported, nor were lesion size, or chronicity of the condition[17].

Strong evidence that PRP might increase systemic and local growth factors to treat T+ in animal models is therefore not available. Like in humans, our hypothesis was that PRP increase growth factors production in an extended way. The aim of our study was thus to quantify the effect of PRP injection on systemic and local growth factors and to identify molecular markers in a rat model of patellar and Achilles T+ treated with PRP.

Materials and methods

The procedure and animal care complied with the "Principles of animal care" formulated by the European Union (Animal Facility Agreement 75-18-03, 2005), and animal experimentation was performed under the authorization **BLINDED** Ministry of Agriculture.

Twenty two immunocompetent male Sprague Dawley rats (providing 88 patellar and Achilles tendons) weighing 250 to 350 g were used for the study. The rats were sedated before and during each manipulation with Isoflurane® (5% for induction and 2.5% for maintenance). Two protocols were used, one to assess the systemic molecular effect of intratendinous injection of PRP in T+ and a second one to assess local molecular effect of PRP in T+.

Protocol 1 (PRP systemic effect):

At Day 0, 10 serum samples of venous blood (jugular puncture) were made on healthy rats to take normal values of systemic biological markers for "baseline" (T-). After, we induced chemical T+ in 20 rats (80 patellar and Achilles tendons) by a single intratendinous injection of Type 1 Collagenase Gibco™ (250 U ie 30 μl, dissolved in 0.09 saline solution PROAM®) using a 29 G needle, under Ultrasound (US) guidance by a single operator. This model of T+ has been described in previous publications and permits one to obtain an animal model of T+ as early as 3 days after collagenase injection and thereafter, up to 12 weeks[18,19].

At day 3, we initiated treatment using either PRP (group 1, n=7 rats) or Physiological Serum (PS, control= group 2 n=7 rats) by the same single operator. Six rats received no injection: they allowed witnesses to the natural evolution of tendinopathy (group 3, n=6 rats).

Treatment consisted of a single intratendinous injection, under US guidance (targeting the thickened segment of the tendon), using a 29 G needle of either 0.1 ml of PRP (PRPT+) or 0.1 ml of PS (ST+). The basic mechanisms for preparing PRP involved withdrawal of the rat's peripheral jugular blood (3 ml) and a single 8 minute spin centrifugation (3000 G) with no activator to obtain a final volume of PRP (visible as a yellow layer) of 1 ml. This PRP obtained had a platelet concentration equal to 3 times (mean =1.500.000±42.000) the concentration measured in the blood as verified using a conventional cytometry method by Scil vet abc Plus®. We chose this concentration as it is the lowest accepted platelet concentration for PRP without activator. Our PRP preparation was also poor in leukocytes to avoid any acute inflammatory response with catabolic effects[20,21].

No specific regimen or restricted activity followed the PRP or PS injection. Figure 1 shows the 3 bottles of supernatant (Platelet Poor Plasma), PRP and red cells, after conventional cytometry method.

To compare the systemic effect of PRP, 3 mL of venous blood serum were collected by transjugular blood puncture (Fig. 2) for systemic biochemical assessment from each rat in each group at each time point (Tab. 1).

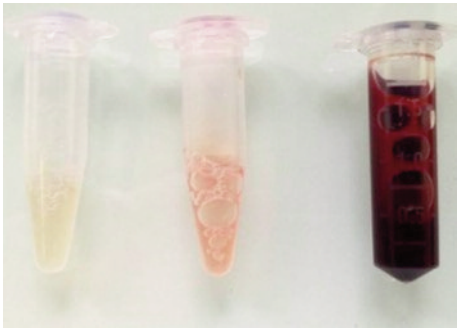


Figure 1. Presentation (from left to right) of the 3 bottles supernatant (Platelet Poor Plasma), PRP and red cells, after conventional cytometry method by Scil vet abc Plus®.

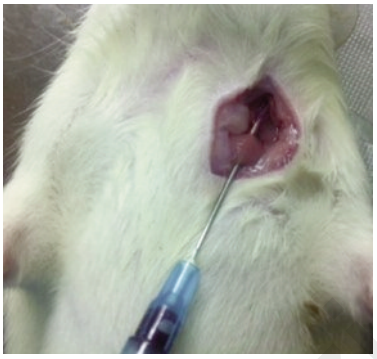


Figure 2. Recurring transjugular blood puncture (3 ml) at day 13 for systemic biochemical assessment.

Table 1. Serum assays were performed on 3 randomly rats selected from the remaining rats not yet sacrificed. One rat per group was sacrificed at each step for the removal of tendons.

		n at D0	D3	D7	D13	D18	D25
Systemic assessment (n=rats)	No injection	6 rats	3	3	3	3	2
	PS	7 rats	3	3	3	3	3
	PRP	7 rats	4	4	4	4	3
Local assessment (n=tendons)	No injection	24 tendons	4 (1 rat)	4 (1 rat)	4 (1 rat)	4 (1 rat)	8 (2 rats)
	PS	28 tendons	4 (1 rat)	4 (1 rat)	4 (1 rat)	4 (1 rat)	12 (3 rats)
	PRP	28 tendons	4 (1 rat)	4 (1 rat)	4 (1 rat)	4 (1 rat)	12 (3 rats)

Protocol 2 (PRP local effect):

PRP local effect was studied by comparing 80 tendons with chemical tendinosis induction (T+) injected with PRP (PRPT+, group 1, n=28, patellar =14, Achilles=14), injected with PS (PST+, group 2, n=28, patellar =14, Achilles=14) or not injected (T+, group 3, n=24, patellar =12, Achilles=12).
At day 3, 7, 13 and 18, one rat (4 tendons) was sacrificed in each group. At day 25, 3 rats were sacrificed in group 1 and 2; 2 were sacrificed in group 3. Biological examination (evaluation of local biological markers) was performed on each sacrificed T+ rat during follow up on sample tendon.
Figure 3 shows removal of left Achilles tendon at day 25 for local biochemical assessment.
Two rats (8 tendons) served as "baseline" and have received no injection of collagenase, PRP or PS. At day 0, they were directly sacrificed to take normal values of local biological markers in healthy patellar (n= 4 T-) and Achilles tendon (n= 4 T-).
All the study design was summarized in Table 1.

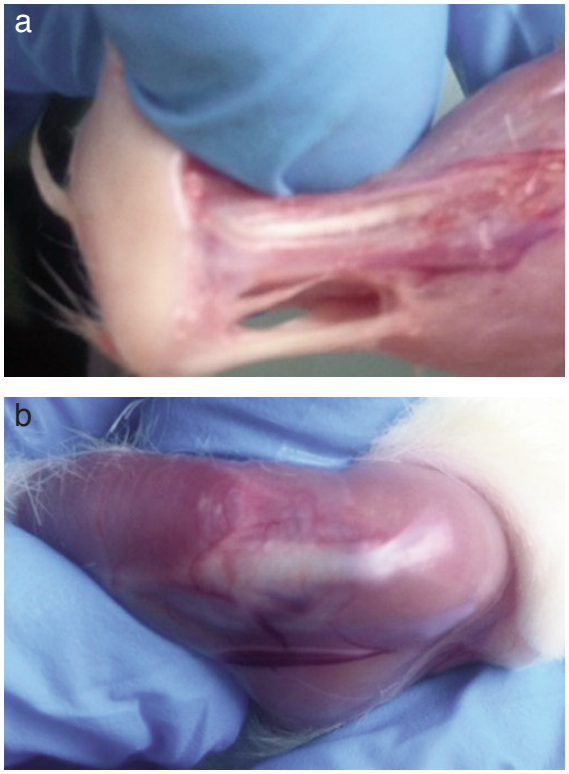


Figure 3. Removal of left Achilles tendon (a) and righ patellar tendon (b) at day 25 for local biochemical assessment.

ELISA method and data biological analysis

Biological evaluation of blood and tendon sample used enzyme-linked immunology assay [ELISA: Bio-Plex ProTM Rat cytokine 24-plex Assay - BIO-RAD TM (order catalog 171-K1001M)] screening method[17] with focus on:

- Ra IL1a, 1b, 2, 4, 5, 6, 7, 10, 12, 13,17, 18.
- Ra EPO, G CSF, GM CSF.
- Ra GRO/KC, IFN-g, M-CSF.
- Ra MIP-1°, MIP-3°, RANTES.
- Ra TNF-a, RA VEGF.

To study the immunological markers on serum sample (protocol 1): a sample (with EDTA: Ethylenediaminetetraacetic acid) from venous blood of 2 ml jugular level has been achieved. It has been a first centrifugation at 2,000 jets for 10 minutes. Plasma itself underwent centrifugation at 2,500 jets for 15 minutes. A tube 100 microliters was individualized for the ELISA assay.
For the study of immunological markers in tendon (protocol 2): Achilles and patellar tendons collected were weighed. Tendons were in RPMI (for 10 milligrams of tendon 100 milliliters of saline are added). The result were incubated 24 hours at 37° C then centrifuged at 3000 jets for 10 minutes. The supernatant was recovered and considered as sample tendon environment. These environments were individualized for assay by ELISA technique.
Operators were blinded to the status of tendons.

Statistical analysis

Statistical analysis was performed using MedCalc® software 11.0.
Comparative series were considered independent of each other. A mean value and standard deviation was calculated for each rat at each control in the 3 groups for each biological marker. To study the effects of PRP, we compared the biological systemic and local markers of PRPT+, PST+, T+ and T- at day 0, day 3, day 7, day 13, day 18 and day 25, using paired t-tests. Then, we assessed the evolution of biological systemic and local markers of PRPT+, PST+, T+ and T- at day 3, day 7, day 13, day 18 and day 25, using a Kruskal-Wallis test.
We considered $p < 0.05$ as significant.

Results

Protocol 1 (PRP systemic effect):

During biological follow up, comparison of all serum samples of PRPT+, PST+ and T+ groups (transversal assessment) showed no significant modification of their biological markers at day 7 (p=0.22), day 13 (p=0.28), day 18 (p=0.83) and day 25 (p=0.64) considering each biological marker and also all subgroups confounded.
Similarly at day 0, day 3, day 7, day 13, day 18 and day 25 (longitudinal assessment), ELISA showed no significant modification of each biological markers in each group T-, PRPT+, PST+ and T+(p>0.73).
Table 2 summarizes mean and standard deviation of each biological systemic sample for PRPT+, PST+ T- and T+ animals at day 0, day 3, day 7, day 13, day 18 and day 25.

Protocol 2 (PRP local effect):

During biological follow up, comparison of immunological sample tendon markers of PRPT+, PST+ and T+ groups (transversal assessment) showed no significant modification of their biological markers at day 7 (p=0.2), day 13 (p=0.58), day 18 (p=0.4) and day 25 (p=0.16) considering each biological marker and also all subgroups confounded.

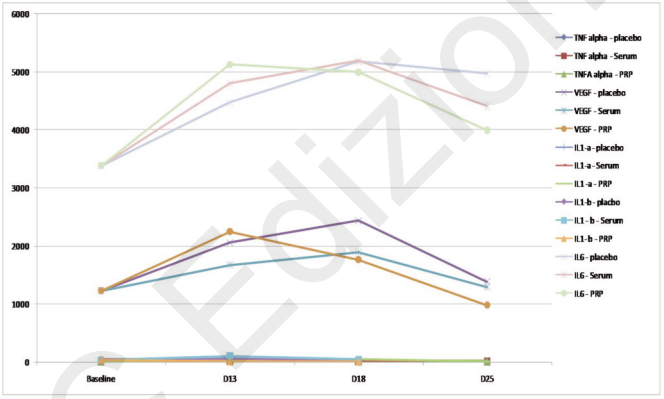
Table 2. Mean and standard deviation of systemic biological markers at day 0, 3, 6, 13, 18 and 25.

Biological Marker			Placebo Group						P Serum Group						PRP Group					
	D0 Baseline		D13		D18		D25		D13		D18		D25		D13		D18		D25	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EPO	133.84	252,84	1447.16	1681.8	733.15	663.73	253.23	390.87	614.72	484.2	532.86	360.5	60.58	25.67	344.17	339.0	894.27	535.7	131.75	260.59
TNF alpha	1.54	3,4	14.25	7.1	22.58	23.73	5.59	5.66	5.11	6.2	27.51	22.6	1.85	0.61	5.18	5.8	9.01	2.5	5.13	2.66
GM-CSF	2.88	1,2	14.75	6.3	10.14	4.37	10.35	10.08	11.84	13.4	44.51	22.2	5.08	4.12	6.65	6.6	13.98	9.0	4.94	3.46
VEGF	5.42	2,24	5.05	1.2	4.46	0.79	4.88	1.45	3.67	3.1	13.48	5.5	2.75	2.21	2.32	2.4	4.44	0.3	3.97	1.68
GRO/KC	15.30	6,58	46.23	44.9	27.15	24.14	40.82	37.99	22.17	33.6	52.48	36.8	17.02	18.15	9.57	3.9	17.44	20.6	6.76	7.55
IL1-a	8.59	6,22	26.06	24.5	7.67	0.82	3.89	4.02	8.60	10.9	78.96	41.0	0.74	0.57	9.56	6.0	6.65	7.1	8.45	1.55
IL1-b	12.54	4,2	32.05	5.9	26.96	6.60	21.26	20.20	25.43	25.4	428.00	280.3	19.03	16.82	17.98	20.7	36.03	18.5	18.80	8.56
IL2	29.80	14,2	42.66	34.4	17.52	11.57	13.06	6.02	24.07	30.4	40.51	34.3	2.53	0.12	11.67	16.8	16.44	14.1	24.11	2.37
IL4	4.79	1,3	3.65	0.4	2.66	1.09	3.26	2.91	7.20	5.5	45.84	30.1	1.35	1.24	3.64	3.0	4.00	2.6	2.59	0.64
IL5	9.48	2,04	49.91	6.8	38.40	6.55	44.14	22.32	31.08	27.9	167.81	63.2	21.73	8.51	21.27	22.4	41.49	21.0	32.60	9.98
IL6	73.14	10,23	113.89	118.7	17.26	11.44	11.93	15.26	25.18	37.7	429.10	237.8	3.52	2.11	32.60	57.9	24.24	35.3	36.76	14.37
IL7	144.93	28,24	204.11	250.3	71.56	85.62	167.66	66.78	59.02	93.9	273.08	211.2	93.42	40.73	143.77	265.8	83.18	57.1	136.90	92.94
IL10	11.95	4,7	47.26	10.0	38.28	15.95	43.44	37.21	36.87	33.8	27.51	9.7	22.61	19.38	23.66	26.2	46.64	20.1	32.80	11.93
IL12p70	5.77	1,25	6.35	2.1	3.97	1.31	5.08	4.58	4.29	5.5	39.45	32.2	1.30	1.86	2.28	2.4	8.66	4.6	5.46	2.98
IL13	5.77	2,33	16.93	6.3	8.83	5.03	9.88	9.46	10.69	11.8	31.99	26.6	5.44	6.17	5.43	4.8	14.70	11.0	5.20	4.72
IL17a	5.20	1,2	4.24	2.4	1.81	1.36	2.30	2.20	2.64	3.3	31.19	17.0	0.64	0.74	0.82	0.8	2.71	2.6	3.08	1.07
IL18	683.73	125,65	6114.29	6378.8	3837.83	3261.36	1880.22	1715.84	2234.72	1922.9	1441.57	875.6	1192.90	1527.41	1159.97	1170.1	3735.13	2379.8	2613.13	1224.72
M-CSF	121.68	24,5	169.21	34.4	185.82	16.30	135.25	7.07	149.77	123.1	157.84	21.2	131.69	26.81	91.76	95.1	193.53	26.0	153.72	69.04
MIP3a	9.91	1,1	8.13	1.3	4.87	1.06	5.99	4.38	4.63	3.9	29.50	12.7	5.58	4.68	3.23	3.2	8.54	2.9	4.32	2.71
Rantes	28.61	9,4	63.68	11.4	27.33	8.93	41.08	32.78	33.50	40.2	25.47	4.7	21.61	14.07	11.78	7.2	31.74	24.1	14.51	11.25

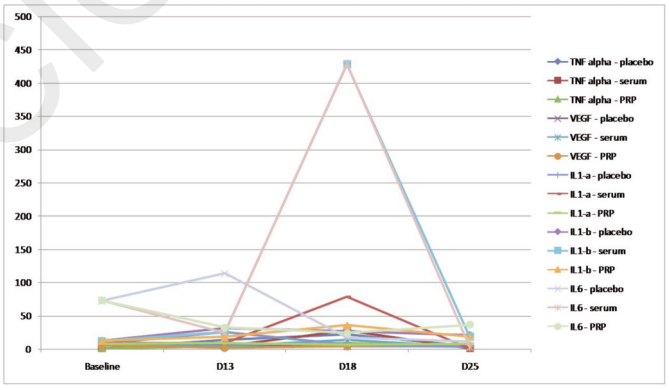
Similarly at day 0, day 3, day 7, day 13, day 18 and day 25 (longitudinal assessment), ELISA showed no significant modification of biological markers in each group T-, PRPT+, PST+ and T+ ($p>0.14$).
Table 3 summarizes mean and standard deviation of each immunological sample tendon markers for PRPT+, PST+ T- and T+ animals at day 0, day 3, day 7, day 13, day 18 and day 25 whereas graphs 1 and 2 focus about main cytokine systemic and local evolution in the different groups.

Table 3. Mean and standard deviation of local biological markers at day 0, 3, 6, 13, 18 and 25.

Biological			Placebo Group						Serum Group						PRP Group					
Marker	Baseline		D13		D18		D25		D13		D18		D25		D13		D18		D25	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EPO	166.320	143.841	369.807	527.974	215.898	225.915	333.893	432.830	864.090	507.957	183.728	128.778	218.405	236.876	240.385	314.332	175.748	124.152	452.474	640.157
TNF alpha	9.152	4.662	26.318	23.592	19.898	4.979	13.125	4.864	21.090	19.223	14.725	6.453	9.774	4.510	16.560	18.245	13.125	4.864	16.709	17.548
GMCSF	3.410	NM	7.170	NM	30.640	35.081	13.845	23.498	3.880	0.396	29.290	39.471	4.865	6.205	12.960	17.423	60.505	30.314	6.092	5.385
VEGF	1226.721	972.574	2053.938	764.059	2434.140	192.399	1379.693	917.481	1670.660	1125.099	1894.103	849.558	1291.697	958.703	2247.333	566.764	1764.808	626.159	979.144	774.459
GRO/KC	1808.886	2116.339	3521.893	887.918	3499.980	NM	3366.344	466.873	3395.210	688.430	3290.083	257.434	3336.338	622.182	3491.687	475.942	3621.025	475.805	2876.287	1118.185
IL1-a	7.490	6.153	10.580	10.761	23.613	12.392	9.238	6.268	10.233	2.015	13.343	14.880	10.361	12.602	12.430	8.292	52.163	70.910	5.635	4.037
IL1-b	NM	NM	44.265	36.597	63.090	55.824	23.163	33.954	32.500	20.597	105.480	NM	39.995	33.568	36.648	21.017	6.510	1.103	10.455	6.747
MCP-1	2431.781	3515.834	9329.505	4330.581	9329.595	1608.891	6492.871	4080.019	6402.095	5434.827	8360.923	2095.054	5400.172	4114.211	11684.44	2550.006	7306.373	4465.130	4339.283	4002.724
IL2	6.008	4.676	29.640	24.025	28.565	13.800	18.740	11.306	22.555	13.907	25.648	19.926	14.222	7.670	21.308	16.007	16.618	7.510	13.905	9.583
IL4	0.693	0.504	4.430	3.324	3.743	2.292	2.256	1.697	3.310	2.746	3.360	2.902	1.887	0.810	3.663	2.597	2.265	1.510	1.024	0.774
IL5	18.053	12.372	41.170	10.808	46.855	11.188	33.754	13.821	39.030	16.682	36.923	13.202	31.208	16.402	49.240	12.060	36.300	13.939	28.321	16.714
IL6	3382.428	1593.634	4473.863	1861.914	5177.540	40.868	4972.757	224.944	4794.693	428.603	5189.320	113.297	4410.127	1231.003	5124.673	226.304	4995.120	292.992	3992.605	1443.205
IL7	50.483	55.732	181.555	290.315	136.763	73.566	105.284	111.574	205.023	244.115	89.643	41.955	82.333	67.999	117.728	149.741	62.890	42.799	149.139	220.943
IL10	71.000	76.712	927.505	900.022	7648.535	13126.66	335.677	283.176	1459.640	1345.801	1512.225	2436.620	245.594	336.567	365.223	365.903	2811.078	5031.938	383.197	504.194
IL12p70	4.890	3.336	12.510	14.439	9.387	10.994	5.796	4.003	NM	NM	15.420	16.292	2.273	3.697	8.165	6.371	1.190	1.344	5.338	3.569
IL13	NM	NM	NM	NM	9.387	10.994	5.796	4.003	1.500	NM	2.810	NM	0.580	0.806	0.943	0.460	0.380	NM	0.500	NM
IL17a	10.470	NM	19.783	20.376	23.820	5.218	15.042	4.075	56.140	74.882	10.110	10.493	3.790	0.953	11.453	10.621	NM	NM	9.970	4.158
IL18	12.280	NM	60.290	75.356	47.355	18.037	54.282	41.625	69.780	62.398	19.573	16.829	16.143	15.020	47.060	43.489	24.173	12.463	47.342	46.832
MCSF	39.010	24.980	44.900	19.554	73.520	27.516	55.278	36.842	34.725	8.391	93.650	52.707	44.063	27.968	65.565	30.374	81.623	31.070	48.152	36.039
MIP-3a	6.313	3.483	95.640	114.332	235.645	277.131	31.935	46.401	66.163	46.940	232.118	91.640	18.723	31.050	49.505	58.016	292.895	552.473	9.694	9.038
Rantes	23.380	10.340	53.480	35.412	45.048	14.652	32.351	9.764	46.618	19.291	38.785	29.174	30.537	5.891	41.263	9.520	78.418	104.966	32.801	10.889



Graph 1. Systemic main cytokines evolution in PRPT+, PST+ and T+ groups (transversal assessment): TNF alpha, VEGF, IL-1a, IL- 1B, and IL-6.



Graph 2. Local main cytokines evolution in PRPT+, PST+ and T+ groups (transversal assessment): TNF alpha, VEGF, IL-1a, IL- 1B, and IL-6.

Discussion

Our study strongly suggests that a single intratendinous US-guided injection of PRP in Achilles and patellar T+ doesn't increase biological markers such as growth factors compared to a control group in mid-term and long-term follow-up. These results are potentially important as, to our knowledge, there has been no study demonstrating the immunological effects of PRP, early in the natural evolution of tendinopathy, before tendon rupture and before the onset of chronic pain.

Reports assessing autologous blood, PRP or varicose vein-sclerosing drugs, injected in different sites (intra, peritendinous) under different conditions (clinically-guided, imaging-guided), in heterogeneous populations, including patients with T+ and patients with rupture, with no long term follow-up and no histological examination[9,22,23] have provided contradictory results. Thus, there is no clear conclusion regarding the curative effect of PRP in T+.

By recently assessing PRP in a rat model [with fixed platelet concentration in PRP (x3) and no adjuvant], with a systematic clinical and US follow-up and histological examination, we have previously provide strong evidence that PRP might be a useful strategy to treat T+[15]. In this study, we used the same setup, animal model, and controlled PRP preparation protocol as described in our pre-clinical model. Based on these results and the potential healing of PRP to stimulate thrombus in tendon as described above, our next step was therefore to assess, after clinical, US and histological evaluation, the biological potential effect PRP to increase growth factor concentration as recently investigated and described in human[17].

Indeed, in literature, intratendinous PRP injection, by locally providing important concentration of active growth factors (PDGF, TGF-β, VEGF...), might promote stem cell recruitment and fibroblast collagen production, and therefore stimulate tendon cicatrization[10]. Moreover, in recent series on human model, Wasterlain AS et al. assayed 6 growth factors by ELISA method in 25 patients before and after intratendinous injection of PRP: human growth hormone (hGH), insulin-like growth factor-1 (IGF -1), insulin-like growth factor binding protein- 3 (IGFBP -3), basic fibroblast growth factor (bFGF or FGF -2), vascular endothelial growth factor (VEGF) and platelet-derived growth factor-BB (PDGF- BB). They showed a significant increase in blood of several growth factors, particularly VEGF which would be the best serum marker of PRP therapy. However, this study, with multiple blood puncture near to the injection of PRP time, only followed the athletes during 96 h (6 noncash between 0.25 and 96 h), with no food or any type of physical exercise 3 hours before each serum sample. His aim was rather to know the short-term effects of injection of PRP for assessing growth factors in the doping. Indeed, PRP treats sports 86,000 athletes in the United States each year and is not considered as doping substance, while growth factors are banned by the World Anti-Doping Agency (WADA).

In our animal experience, the first serum assays were beginning 72 h post PRP injection with a long term follow up of 25 days without limitation in terms of food or exercise for animals, in order to correlate possible changes in serum phases of tendon repair, but even early assays at day 3 showed no difference. In another animal study performed on horses, McCarrel TM et al.[21] demonstrate a lower level of expression of interleukin 1 beta and TNF alpha in tendons treated with a low concentration of leucocytes in PRP compared to intermediate and high levels. This could explain in part our results concerning IL 1 and TNF alpha, in comparison with Wasterlain et al. in which the platelet or leukocyte counts in the PRP treatment were not reported.

Moreover, we didn't find any significant changes in the evaluated biomarkers after collagenase injection during the natural evolution of T+ in the untreated group. We also highlight an interesting to know the important inter-individual variation for all interleukins and growth factors at day 0 (baseline) in the physiological state, without inducing element tendinopathy. Indeed, we observed very different values, including VEGF ranging from non-detectable to important value (Tab. 2). These very different inter-individual perspective core values could be an answer as to the ineffectiveness of PRP in some individuals. A dosing study of physiological values of growth factors and interleukins humans (in a non-patient population) to search for possible differences might be interesting to explain some differences in efficiency, according to the Authors, protocols and series.

Our *in vitro* study suffers from three biases: first, we measured prostaglandins, interleukins and growth factors directly in the serum using ELISA, instead of the more commonly used method that indirectly measured RNA with RT-PCR[24]. We choose the ELISA method as we had been using it since many years in our laboratory where the method was validated with internal controls and used in the work of several publications[25,26]. Second, twenty-two rats were included in our study but due to the iterative sacrifices to allow the collection of tendons during follow-up, only 3 rats in the group without injection as well as in the group treated with PS and only 4 in the PRP group were taken at each step. Although this population was sufficient for statistical calculation, additional studies including a larger number of rats will be needed in the future to confirm our preliminary results. Third, we did not focus on short term biological effect of PRP and an earlier measurement should be performed in future studies. Such results necessitate however additional studies before the protocol can be applied to human patients[27].

Conclusion

Our study suggests that a mono-injection of PRP in tendinosis using a controlled concentration of platelets and leukocytes doesn't increase biological markers such as growth factor in rats in mid-term and long-term follow-up, according to ELISA criteria. Biological

studies, prospectively comparing PRP to placebo control group should be initiated in the future.

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