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RESEARCH ARTICLE

A NEW PROTOCOL FOR OBTAINING PLATELET RICH IN GROWTH FACTORS (PRP). A DESCRIPTIVE STUDY IN 15 PATIENTS AND COMPARISON WITH RESULTS PUBLISHED IN LITERATURE

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ABSTRACT

INTRODUCTION: The diversity of procedures for obtaining platelet and plasmatic growth factors, the absence of control in most of them and the growing field of clinical application, makes them necessary methods adequately structured, documented, controlled and tested, playable by any author. The present series of clinical cases aims to introduce and test a specific technique for obtaining PRP, with precise characteristics both production and final composition of compound got, in 15 hematological healthy patients, comparing our results with those obtained by other procedures scientifically tested.

MATERIAL AND METHODS: 15 caucasian patients were selected , 8 male and 7 female with age range between 35 and 65, healthy haematologically. The procedure for obtaining the PRP, consisted of a single centrifugation of the blood sample for 30 minutes at 3500 rpm in a angular shaft of 16 tubes centrifuge serie (CEMCON 2) and micropipetting the protein fraction rich in platelet and plasmatic growth factors and cell through open technique under aseptic conditions in horizontal laminar flow hood Grade A at a temperature of 22 ° C, with the use of leuco-platelet or Buffy-coat layer (PRP rich in leukocytes).

RESULTS: No correlation between the amount of concentrated platelets and the amount of growth factors finally obtained was observed. The protocol set forth concentrated levels of platelets and leukocytes approximately 3 to 5 times higher than baseline levels with a predominance of mononuclear. Levels of growth factors from 7-10 times greater than the patient's baseline levels, with little variation in them. The growth factor levels were stable in the blood of each patient within 24 h of treatment between 7 and 9 times higher compared to the previous baseline. Compared with other procedures discussed in the literature; This method achieves concentration between 1.5 and 3 times more platelets in the final product, with a purification of growth factors overall type VEGF and TGF-B clearly superior.

CONCLUSION: the technique disclosed is more effective since concentrate achieves greater amount of platelets and growth factors and efficient since it maintains a serum protein in these stable sera of patients after 24 hours of administration thereof.

INTRODUCTION

The use of platelet growth factors, or what is commonly known as Platelet-rich plasma (PRP), is becoming a technique considered medication, despite sharing features of the autotransfusion, with multiple clinical applications in different fields of medicine: trauma and sports medicine, dentistry and maxillofacial surgery, plastic surgery and burns, dermatology, Neurology and Neurosurgery etc. The diversity of existing procedures, the absence of control in most of them and the growing field of clinical application, makes them necessary methods adequately structured, documented, controlled and tested, playable by any author in view to carry out monitoring and traceability of the final product, its possible therapeutic effects as well as side effects that could occur. Nor are there any scientific works in the literature showing immediate monitoring of growth factors in serum of patients, in order to make a proper traceability effect thereof. It is the closest thing to the elaboration of a sheet of the concentrate produced in order to guarantee maximum efficiency and safety for the patient. (1, 2 and 3). The present work, series of clinical cases, aims to introduce and test a specific technique for obtaining PRP, with precise characteristics both production and final composition of compound got, in 15 hematological healthy

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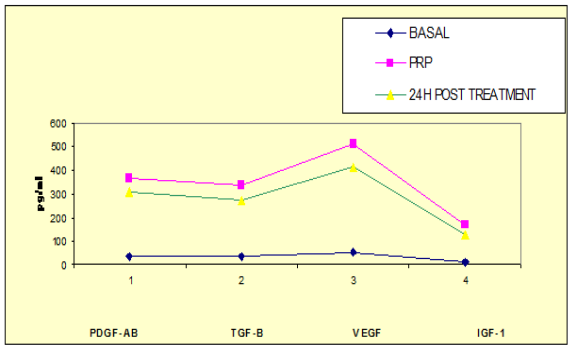
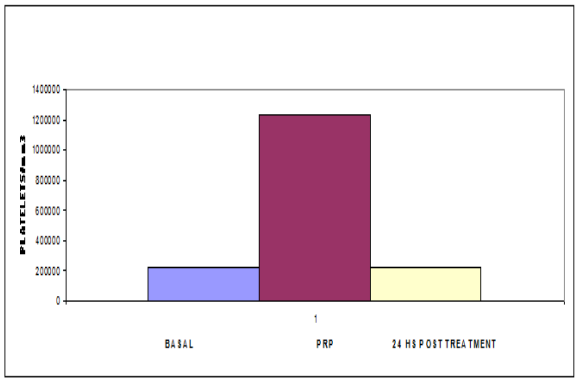


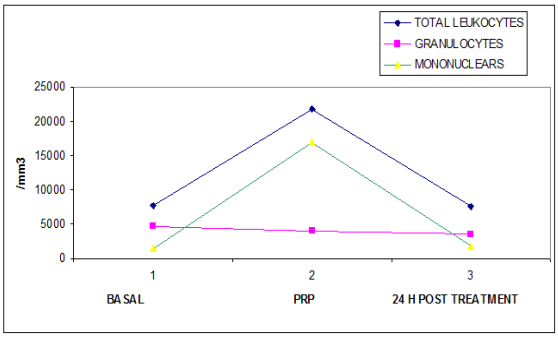
Growth factor levels were stable in the blood of each patient within 24 h of treatment between 7 and 9 times higher compared to the previous baseline

Figure 1. Average levels of growth factors in Alcaraz, Oliver and col technique measured at baseline, PRP and 24 h after the treatment



Method concentrated levels of platelets aproximately 3 to 5 time higher than baseline

Figure 2. Half platelet counting in Alcaraz, Oliver *et al* technique at baseline, PRP and 24 h after treatment

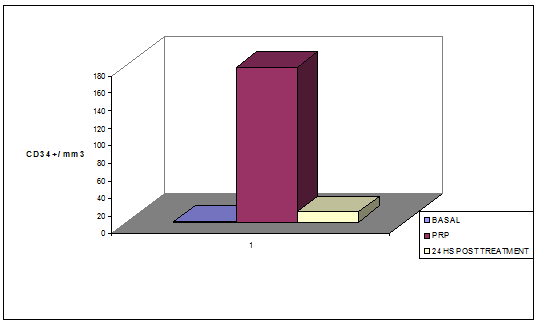


Protocol of treatment obtains levels of leukocytes between 3 and 5 times higher than baseline levels with a predominance of mononuclears

Figure 3. Half leukocitary (granulocitary and mononuclear) counting in Alcaraz, Oliver and col technique at basaline, PRP and 24 h after treatment

MATERIALS AND METHODS

15 caucasian patients were selected, 8 male and 7 female with age range between 35 and 65, healthy haematologically following analytical standards autologous inclusion of the Spanish Society of Hematology and Hemotherapy regarding biochemical, hematological and serological before obtaining samples of whole blood controls. A closed system blood by Vacutainer tube connected to 3.5ml EDTA was used.



Changes in cell counts practically safe increase between 150 and 300 times higher in the determination of CD 34 + at 24 hr post administration of PRP baseline count

Figure 4: Half CD34+ counting in Alcaraz. Oliver and col technique at basaline, PRP and 24 h after treatment

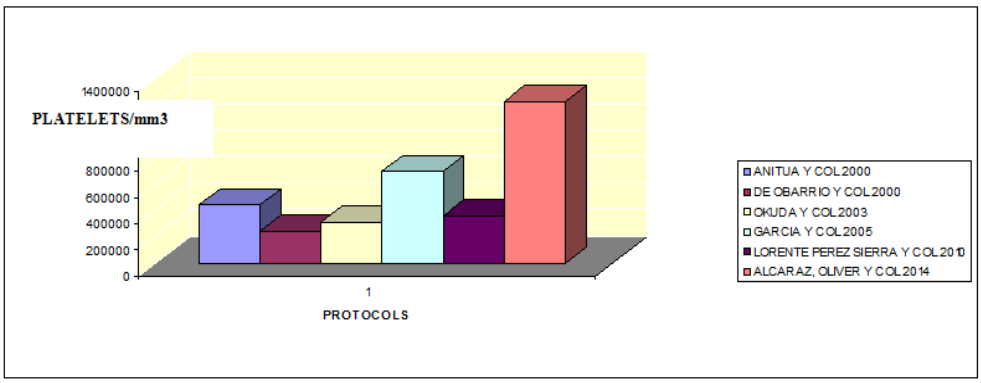
Forearm venous access was used 20-gauge needle for adults and 22 G for children. The procedure for obtaining the PRP, consisted of a single centrifugation of the blood sample for 30 minutes at 3500 rpm in a angular shaft of 16 tubes centrifuge serie (CEMCON 2) and micropipetting the protein fraction rich in platelet and plasmatic growth factors and cell through open technique under aseptic conditions in horizontal laminar flow hood Grade A at a temperature of 22 ° C, with the use of leuco-platelet or Buffy-coat layer (PRP rich in leukocytes). In the final product, we proceeded to cell counting by hemocytometer Coulter (Beckman), platelets, leukocytes, granulocytes, monocytes and CD 34 + / mm3 and the following platelet growth factors: growth factor derived from platelets (PDGF), transforming B factor (TGF-B), Insulin like-1 growth facto (IGF-1) and vascular endothelial growth factor (VEGF) using specific kits of enzyme-linked immuno-assay (ELISA). Measurements were made at baseline, prior to treatment, and serological PRPs obtained in patients 24 h after administration. The route of injection was varied: intraarticular, intravenous, intramuscular or subcutaneous. The most consolidated techniques in literature for obtainig PRP (Anitua *et al*, De Obarrio *et al*, Okuda *et al*, Garcia *et al* and Sierra Perez Lorente *et al*) were utilized to compared their performance with ours: (3 and 4). Descriptive statistical calculations for the interpretation of analytical data were applied in each case.

RESULTS

In Tables 1, 2 and 3 you can see the results for the 15 patients in terms of cell count and levels of growth factors obtained with the maximum and minimum values collected, and the average parametral achieved both at baseline as in the PRPs and blood at 24 h of treatment for each patient. No correlation between the amount of concentrated platelets and the amount of growth factors finally obtained was observed. The protocol set forth concentrated levels of platelets and leukocytes approximately 3 to 5 times higher than baseline levels with a predominance of mononuclears (75-90% of the white cellularity), up to 1-3% of them positive for marker CD 34+ without examining changes in cell counts practically safe increase between 150 and 300 times higher in the determination of CD 34 + at 24 hr post administration of PRP baseline count, as can be seen in Figures 2, 3 and 4 respectively; and a levels of growth factors from 7-10 times greater than the patient's baseline levels, with little

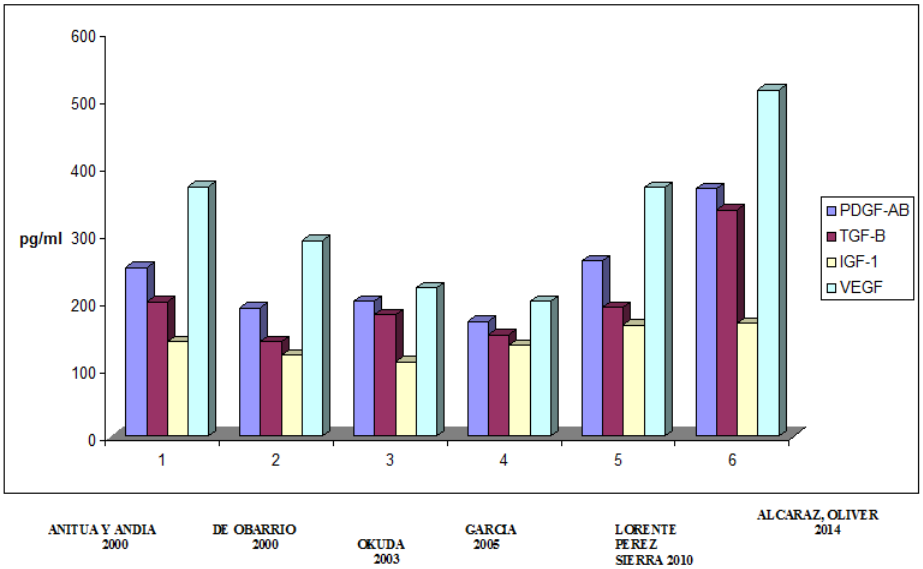
variation in them. The growth factor levels were stable in the blood of each patient within 24 h of treatment between 7 and 9 times higher compared to the previous baseline, visible in Figure 1, Compared with other procedures discussed in the literature;

This method achieves concentration between 1.5 and 3 times more platelets in the final product, as we can see in Figure 5, with a purification of growth factors overall type VEGF and TGF-B clearly superior, visible in Figure 6.



Compared with other procedures discussed in the literature; This method achieves concentration between 1.5 and 3 times more platelets in the final product

Figure 5. Average count of platelets in the PRPs of 6 procedures examined



Compared with other procedures discussed in the literature; This method achieves purification of growth factors overall type VEGF and TGF-B clearly superior

Figure 6. Average concentration of growth factors in the PRPs of 6 procedures examined

Table 1. Rheological caracteristiques of blood patients at baseline:

	PDGF-AB (10-50 pg/ml)	TGF-B1 (10-70 pg/ml)	IGF-1 (0,5-19,5 pg/ml	VEGF (15-85 pg/ml)	Platelets (150.000-350.000/mm3)	Leukocytes (3.200-9000/mm3)	Granulocytes /mm3	Mononuclears /mm3	CD 34 + /mm3
Patient 1	45	60	18	80	210000	7500	4875	1275	0.9
Patient 2	40	25	10	45	210000	6500	3575	1625	0.3
Patient 3	43	55	17	80	190000	6230	3738	1246	0.4
Patient 4	43	67	15	75	170000	7500	4500	1125	0.5
Patient 5	15	25	7	30	180000	8900	5340	1335	0.3
Patient 6	35	24	12	40	175000	8900	5340	1956	0.2
Patient 7	20	15	7	30	260000	7200	4320	1440	0.2
Patient 8	30	20	7	35	176000	7430	4458	1114	0.4
Patient 9	91	60	16	75	350000	7430	4086	1337	0.7
Patient 10	45	55	18	70	195000	9500	5700	1425	0.7
Patient 11	35	20	15	40	205000	8300	4980	1909	0.2
Patient 12	12	15	4	25	250000	8500	5100	1890	0.1
Patient 13	45	60	17	75	240000	8700	5481	1131	0.7
Patient 14	43	55	17	70	300000	7600	4560	1140	0.4
Patient 15	15	55	18	70	210000	7500	4500	1500	0.2
Maximum	91	67	18	80	350000	9500	5700	1956	0.9
Minimum	12	15	4	25	170000	6230	3575	1114	0.1
Average	32,74	35,02	11,58	50,94	219675	7782	4645	1411	0.3

Table 2. Rheological characteristics of PRPs obtained by the tested procedure:

	PDGF-AB (10-50 pg/ml)	TGF-B1 (10-70 pg/ml)	IGF-1 (0,5-19,5 pg/ml	VEGF (15-85 pg/ml)	Platelets (150.000-350.000/mm3)	Leukocytes (3.200-9000/mm3)	Granulocytes /mm3	Mononuclears /mm3	CD 34 + /mm3
Patient 1	496	450	250	575	1200000	21000	3150	18270	240
Patient 2	370	300	150	545	1270000	22000	4400	16500	180
Patient 3	390	370	200	590	1270000	21000	3150	16800	270
Patient 4	450	480	190	540	1280000	20000	4000	17400	210
Patient 5	250	265	110	460	1200000	21000	4200	14700	170
Patient 6	360	270	160	530	1220000	24000	6000	19200	175
Patient 7	300	250	120	470	1250000	21500	4515	15910	170
Patient 8	350	235	105	390	1230000	21500	3440	12900	120
Patient 9	553	520	277	590	1230000	21500	4085	18705	215
Patient 10	420	470	210	590	1270000	24000	3600	20400	200
Patient 11	300	270	160	480	1190000	23000	4600	17940	150
Patient 12	190	150	90	320	1200000	20000	4000	12000	70
Patient 13	480	420	230	570	1210000	22000	3300	18700	200
Patient 14	450	420	199	570	1250000	22000	4400	16500	185
Patient 15	345	430	190	590	1270000	23000	4370	19550	200
Maximum	553	520	277	590	1280000	24000	6000	16860	176
Minimum	190	150	90	320	1190000	20000	3150	20400	270
Average	367,42	335,16	167	513,6	1235623	21800	4023	12000	70

Tabla 3. Blood serum features in patients at 24h of treatment.

	PDGF-AB (10-50 pg/ml)	TGF-B1 (10-70 pg/ml)	IGF-1 (0,5-19,5 pg/ml	VEGF (15-85 pg/ml)	Platelets (150.000-350.000/mm3)	Leukocytes (3.200-9000/mm3)	Granulocytess /mm3	Mononuclears /mm3	CD 34 + /mm3
Patient 1	390	375	200	499	270000	8100	3200	1950	20
Patient 2	395	260	103	430	230000	6300	3100	1800	10
Patient 3	290	290	170	505	205000	7500	3500	1900	15
Patient 4	305	385	155	476	190000	8400	3400	2000	15
Patient 5	230	220	95	390	195000	3950	3950	1750	7
Patient 6	280	270	101	410	210000	8700	3600	1700	7
Patient 7	240	240	100	400	235000	7900	3200	1900	15
Patient 8	270	165	78	295	205000	7950	3750	1600	7
Patient 9	480	450	200	460	290000	8450	3900	1600	10
Patient 10	370	250	170	495	205000	7800	3700	1700	15
Patient 11	330	220	100	400	230000	8400	3600	1750	12
Patient 12	135	120	55	200	230000	8600	3900	1600	5
Patient 13	370	350	195	490	245000	8900	3930	1900	15
Patient 14	390	370	170	450	190000	7200	4000	1700	16
Patient 15	310	350	150	485	250000	8300	3900	1750	15
MAXIMUM	480	450	200	505	290000	8900	4000	2000	20
MINIMUM	135	120	55	200	190000	3950	3100	1600	5
AVERAGE	306,8	272,7	127,3	415,45	223602	7638	3629	1768	11

DISCUSSION

Although in this study of case series, the number of patients of the sample is small, the proposed method focuses a greater amount of platelets as compared to other treatment protocols consulted if not correlated with the amount of growth factors obtained regardless of the age and sex of the patient (3 and 4), Consistent with the above previously by other authors. If it is noteworthy that in our PRPs that are rich in leukocytes there is greater amount of platelets and growth factors overcoat TGF-B and VEGF, compared to other procedures in which the leukocyte-depleted fraction has the final product obtained, as already proposed in previous studies, which can be explained by the presence of as many platelets in the border area leuco-platelet spinning. (5 and 6). Another fact to note is the presence of levels of platelet and plasma factors stable growth at 24 h post-treatment blood of very similar to the corresponding PRPs focused on patients, which could be explained by the high capacity of diffusion through of different tissues having these proteins, regardless of the means used to manage (3). Similarly, review the mobilization capacity they have on CD 34+ (4 and 5), detecting blood higher figures at 24 h post-application compared to baseline count.

Adequate studies in translational medicine are still needed to corroborate these results and correlate clinically with those medical applications where it really be useful.

Conclusion

Compared to other methods of obtaining platelet and plasma factors growth reviewed in the literature, the technique disclosed is more effective since concentrate achieves greater amount of platelets and growth factors and efficient since it maintains a serum protein in these stable sera of patients after 24 hours of administration thereof.

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My wife and my son, for his patience and dedication, for their encouragement and courage, without which it would not have been possible to implement this article

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