

PDGF and VEGF Levels in Platelet-Rich Plasma

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Abstract: PDGF and VEGF are two of the most potent mitogen for connective tissue, its secretion appears to be particularly important when the source is Platelet Rich Plasma (PRP), hence the latter leading role in tissue regeneration. ELISA PDGF_B levels in PRP, Platelet Poor Plasma (PPP) and exudates, were determined in 32 healthy subjects before and 24 hours after ingestion of Aspirin (ASA) and Clopidogrel (CLO). Results: PDGF_B baseline levels were 10.6 ± 1.9 ng / ml (PPP), 12.12 ± 2.5 ng / ml (PRP) and 10.84 ± 1.68 ng / ml (exudate) While after treatment with PDGF_B ASA concentrations were at 8.96 ± 1.4 ng / ml (PPP), 11.36 ± 1.48 ng / ml (PRP), 11.11 ± 1.14 ng / ml (exudate) and the Clopidogrel were 8.53 ± 0.59 ng / ml (PPP), 9.65 ± 1.17 ng / ml (PRP) and 8.51 ± 0.75 ng / ml (exudate) . VEGF basal values were 973.9 ± 590.3 pg / ml (PPP), 1184.2 ± 288.4 pg / ml (PRP), 1069.3 ± 192.3 pg / ml (exudate). After treatment with ASA VEGF values were at 1439.5 ± 117.4 pg / ml (PPP), 1802.3 ± 123.9 pg / ml (PRP), 1745.6 ± 171.6 pg / ml (exudate) and the Clopidogrel were 577.8 ± 150.6 pg / ml (PPP), 668.7 ± 100.6 pg / ml (PRP), 624.9 ± 106.7 pg / ml (exudate). Conclusions: It was noted that after administration of antiplatelet drugs PDGF_B and VEGF values decreased statistically significantly, especially for the group of Clopidogrel. The ASA lesser extent appear to affect the concentrations of both factors, which may be attributable to the pharmacological action mechanism between the ASA and Clopidogrel.

Keywords: PRP, PDGF, VEGF, ASA, Clopidogrel.

INTRODUCTION

Platelets are packed with secretory granules, which are necessary to fulfill their functions. Among the three types of secretory granules are α granules, dense granules and lysosomes, or bodies, of which the most abundant are α granules, which have an important content of protein, soluble mediators such as cytokines, growth factors (GF), between the latter proteins are pro and anti-angiogenic factor including vascular-endothelial growth (VEGF), epidermal growth factor (EGF), a growth factor derived from platelets (PDGF), growth factor FGF fibroblast, hepatocyte growth factor (HGF), and the like growth factor (IGF), among others. All these additional FC are secreted by a variety of cells involved in the inflammatory process, but the rate at which platelets accumulate at sites of vascular injury makes them especial mitogenic mediators [1].

Among the preparations that isolate and concentrate platelets, there is Platelet Rich Plasma

(PRP), which has been recognized as a powerful hemostatic and adhesive agent from the 1970s [2], as well as a potent source of GF since 1990 [3]. The PRP is a plasma fraction which has a higher platelet concentration of 2 to 5 times the number of platelets in peripheral blood and is considered PRP platelet concentrate, usually obtained by centrifuging blood from the patient to whom we will apply (autologous). This compound is natural GF carrier, both in plasma and in the unactivated calcium activated or thrombin; the result is a fibrin clot containing in addition to cell adhesion molecules (fibronectin and vitronectin) and GF, involved in coagulation process, wound healing and tissue regeneration [4-7].

It has been shown that the growth factors released after a determined stimulus α granules of platelets, have the property to induce mitogenesis (increasing the number of cells involved in tissue repair) control the release of other growth factors cells that promote synthesis of fibroblasts and osteoblasts, accelerate the effects of growth factors from other cells, under the platelets have a shelf life of 10 days and therefore its action is to be continued by other cells such as macrophages, which are capable of releasing growth

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factors. Among the most studied soluble mediators that are released from the PRP, are epidermal growth factor (EGF), growth factor, platelet derived (PDGFAB) and vascular endothelial growth factor (VEGF) and interleukin 4 (IL -4) and 6 (IL-6) [8].

The first studies that supported the medical use of PRP were made in the field of odontology, as it considered a rich source of growth factors that modulate cell proliferation, leading to successful periodontal tissue regeneration [9-11].

Then, other branches of medicine took these experiences to justify the use of PRP in other conditions, as shown in patients with hemophilia A, hemophilia B, lower extremities ulcers in diabetic patients, arthritis, and other diseases [12].

The main role of PDGF is to promote chemotaxis; is secreted by platelets in the the injury site of fracture and can stimulate recruitment, proliferation, and survival of mesenchymal cells, smooth muscle cells, endothelial cells, fibroblasts, and other repair cells, likewise, the complex initiates capillary angiogenesis in procedures involving the placement of a graft. The mitogenic effect on osteoblasts is a reflection of its primary action in bone [10, 13, 14].

VEGF also called vascular permeability factor (VPF) is the most powerful and ubiquitous of known vascular growth promoters, presenting five different isoforms; the most abundant isoform in platelets is the VEGF-A. Among the factor producing cells there are platelets, macrophages, osteoblasts and smooth muscle cells mainly in hypoxic state. Acts on tyrosine kinase receptors in endothelial cells and is considered a potent angiogenic. [15-20]. Now, it is widely, use drugs that have antiplatelet effect on platelets as acetylsalicylic acid Aspirin (ASA) and Clopidogrel / Ticlopidine (Thienopyridines), among others, whose indications are universal in treating patients with a tendency to form thrombi by various causes such as congenital or acquired heart disease, single or multiple episodes of stroke, among other diseases alpha [21].

In many cases the treatment of various dental conditions leads to the loss of teeth or bone support, and consequently progressive resorption occurs with alveolar bone loss, making difficult the task of reparation for the morphological and functional changes caused by the pathogenesis of each situation. In this context, it has been researching and using platelet-rich plasma to achieve not only the repair of surgical wounds but also for regeneration of lost tissue [22].

In that sense, it is disclosed that the use of PRP and PRF provide a new and useful therapeutic tool in accelerating wound healing and bone maturation and maxillofacial reconstructive surgery. In this regard, Marx *et al.* [23] and Fennis *et al.* [24] demonstrated that the PRP may improve bone regeneration and that platelets can act as local regulators of the healing process, in turn, the application of PRP and the FC that contains increased microcirculation gingival mucosa surrounding the wound.

As above stated, on the mechanism of action and therapeutic effect of antiplatelet drugs in various medical conditions and the use of PRP to accelerate tissue regeneration and repair, this study aims to determine PDGFBB and VEGF levels in PRP and its products in healthy subjects treated with antiplatelet agents and their correlation with the platelet account. And determine whether prophylactic use of such drugs can significantly alter the concentration of growth factors in the PRP, and if not, possible to recommend the use of PRP in patients for whom these drugs are indicated in order to reduce the risk of forming clots.

MATERIAL AND METHODS

The design of this study is Experimental and Longitudinal [25]

The study population was made up of all adult subjects of both genders, healthy in appearance, who attended the Clinical Research Institute "Dr. Américo Negrette "of the Faculty of Medicine, University of Zulia. This population is finite in nature, i.e. less than 100 thousand subjects [25]

The sample was calculated and intentional not random [25]. Corresponded to 32 subjects. The selection criteria were taken into count was:

Inclusion

- Age between 18 and 50.
- Fasting.
- No known underlying medical illness.
- Apparently healthy.
- Normal results in the platelet aggregation studies to be carried out before ingestion of antiplatelet drugs to be used in this study.

Exclusion

- Having ingested antiplatelet drugs 11 days before the study.

To minimize slant in the sample, the sampling technique used corresponded to the first 32 subjects who met all inclusion criteria and willing to participate in this study.

All subjects underwent clinical examination and comprehensive laboratory, in order to rule out systemic disease. These were distributed as follows:

- Group A: 32 subjects without pretreatment with antiplatelet drugs. These same formed the Group B.
- Group B was divided into two subgroups:

B1: consisting of 16 subjects who received aspirin at a dose of 100 mg once a day.

B2: Clopidogrel 16 individuals who received a dose of 75 mg in a single dose a day.

Subjects both group A and B (after 24 hours of consumption of the respective drug) fasting prior aseptic zone 16 mL was extracted antecubital venous blood, it was used for scalp No 21 using the technique of dual syringe to prevent platelet activation.

The first syringe containing 2.5 mL of blood was dispensed into glass tubes containing EDTA to study Hematology, collecting data relating to platelets and white cells, using an automatic cell counter Beckman Coulter AC-T.

The second syringe containing 13.5 mL of venous blood, which was distributed as follows:

1. 4.5 mL was dispensed in a plastic tube containing sodium citrate 3.8% and centrifuged at 800 rpm (180 g) for 10 minutes at room temperature to obtain PRP, and then the remainder of each sample was centrifuged at 4500 rpm for 20 minutes in a refrigerated centrifuge (Sorvall) to obtain platelet poor plasma (PPP). To make platelet aggregation (only before treatment with antiplatelet drug), the platelet concentration was set to $250 \times 10^9 / L$ with the PPP. The aggregation is performed according to the turbidimetric method of Born [26] using a Chrono-log aggregometer (Haverton Corp., PA, USA). The result is expressed as a percentage.
2. 9 mL of sample was placed in another plastic tube containing 1 ml of sodium citrate 3.8% and

were subjected to centrifugation in a clinical centrifuge at a speed of 1400 rpm for 7 minutes at 267 G and following the technique of Anitua [21] to obtain PRP. Total volume obtained in PRP was removed 0.5 mL which corresponded to Platelet Poor Plasma (PPP). The rest (PRP) was taken with meticulous pipetting with a different tip, to the area located above the red fraction; 1 ml total volume was aliquoted for PRP platelet counts and measurement of FC. A 1 ml of PRP obtained was added 50 μ l of Calcium chloride 10% (CaCl₂), mixed and allowed to stand for 15-30 minutes to obtain PRP gel, this was subjected to centrifugation at 2500-3000 rpm for 10 minutes to obtain an exudate (Ex)

3. The PPP, without activating PRP and PRP gel exudate were distributed in aliquots and stored in Eppendorf plastic tubes at -70°C ° in upright deep freezer (Forma Scientific U95-18), where is preserved until FC analysis, technique using Enzyme Immunoassay (ELISA) [27].
4. PDGFBB and VEGF levels were measured in samples and standards using the ELISA kits were supplied by whose Abcam (Abcam Inc., Cambridge, USA: 1 Kendall Square, Ste B2304 Cambridge, MA 02139-1517 USA) with batch number: 85403-1 GR, GR GR85404-1 and 56644-1, were executed according to the instructions of the MANUFACTURER.

Reference Values

We considered the reference values for platelets between $150000-450000 \times \text{mm}^3$ in peripheral blood [28].

For tabulation and analysis of the results obtained, the respective statistics were used. The data shown in tables and graphs (as found) in absolute values and percentages as well as mean + / - 1 standard deviation. For comparison of the study variables used Student's t test was considered $p < 0.05$ as the lowest probability and for the study of correlation Pearson test was used [29].

All subjects were required written informed consent before inclusion in the study, and are identified by numbers, was told also, with the approval of the Ethics Committee of the institution (Institute of Clinical Research, Faculty of Medicine, University of Zulia) and proceeded according to the principles of the Helsinki Declaration of 1975 (updated in 2000), and the

recommendations made by the Council for International Organizations of Medical Sciences (CIOMS) in 2002 [30].

RESULTS

Table 1 shows a comparison of the mean values at baseline and after treatment with ASA / Clopidogrel PDGFBB and VEGF obtained from the subjects studied.

PDGFBB baseline levels ranged on average and standard deviation 10.68 ± 1.9 ng / ml, 12.12 ± 2.51 ng / ml and 10.84 ± 1.68 ng / ml for the PPP, PRP and exudate respectively vs 8.96 ± 1.4 ng / ml, 11.36 ± 2.48 ng / ml and 11.11 ± 1.14 ng / ml after treatment with ASA. Statistically significant differences (p <0.001) only for the PPP, but not to the PRP and exudate. Besides a decrease PDGFBB levels in two of the three platelet products 24 hours after treatment.

It also shows the comparison of the concentrations of PDGFBB for PRP and its byproducts before and after the administration of Clopidogrel. Mean levels and standard deviation measured growth factor after treatment were at 8.53 ± 0.59 ng / ml (PPP), 9.65 ± 1.17 ng / ml (PRP) and 8.51 ± 0.75 ng / ml (exudate). By comparing these values with the baseline was noted a statistically significant decrease in the levels of PDGFBB on all products (PPP and exudate: p <0.0001, PRP: p <0.0005).

With respect to baseline levels of VEGF were found in PPP: 973.9 ± 590.3 pg/mL, PRP: 1184.2 ± 288.4 pg/ml and exudate: 1069.26 ± 192.3 pg/ml. When compared with GF levels obtained after treatment with ASA and clopidogrel, no significant differences were observed as far as the ASA group refers to any of the products analyzed. Unlike what obtained for Clopidogrel where statistically significant differences

were found for all three PRP's derivatives p<0.001 (PPP) and p<0.005 (PRP and exudates respectively).

DISCUSSION

PRP contains growth factors as PDGFBB, EGF, VEGF, IGF-1, TGF-β1, among others, most of them result in the secretion of α granules of platelets during activation. The PDGFBB is one of the most potent mitogen effective action on the connective tissue. Its secretion during platelet aggregation and coagulation can stimulate chemotaxis and cell multiplication, hence leading role in tissue regeneration [31].

Other studies have shown that a single application of a recombinant factor 20pm type PDGF-BB, can achieve a significant effect on the increase in capillary density. A similar effect could be achieved in patients treated with PRP [32].

In the field of periodontics, use of PRP has been described as an adjuvant of regenerative therapy. Some authors found a significantly great increase in periodontal ligament when injured sites were treated with PRP [33,34].

In the field of implantology, reported the use of PRP in preparing the jaw bone for implant placement as well described in the alveoli which are placed PRP show greater buccolingual / palatal bone width, accompanied by increased bone density and a faster tissue coverage, compared with patients who did not use this compound [28,36].

When comparing the concentrations determined in the PRP PDGFBB and its derivatives before and after treatment with aspirin / clopidogrel (Table 1), with respect to the group of AAS, significant differences were found for the PPP (p <0.001) but not for the PRP and exudate, prevailing in significantly lower GF levels

Table 1: PDGF and VEGF Levels in Healthy Subjects and Derivatives PRP Before and After Ingestion of Aspirin and Clopidogrel

	Growth Factors Levels									
	PDGF (ng/ml)					VEGF (pg/ml)				
	Before	ASA	p	C	P	Before	ASA	P	C	P
PPP	10,68±1,9 (8,5-15,2)	8,96±1,4 (7,6-11,1)	<0,001	8,53±0,6 (7,5-9,2)	<0,0001	973,9±590,3 (365,2-1809,3)	1439,4±117,4 (286-2766,3)	NS	577,8±150,6 (382,4-1192,2)	<0,001
PRP	12,12±2,5 (9,2-16,9)	11,36±2,4 (9,0-17,7)	NS	9,65±1,1 (8,0-11,5)	<0,0005	1184,2±288,4 (378,9-1988,8)	1802,98±723,9 (478,9-2828,7)	NS	668,7±100,6 (389,31-1471,1)	<0,005
Exudado	10,84±1,6 (9,2-13,7)	11,11±1,1 (9,0-13,13)	NS	8,51±0,7 (6,7-9,7)	<0,0001	1069,26±192,3 (330,7-3446,7)	1745,63±771,6 (706,3-3267,3)	NS	624,9±106,7 (334,2-1288,5)	<0,005

NS: No significative; ASA: Acido Acetil Salicílico (Aspirin); C: Clopidogrel.

in 2 of the 3 products (PPP and PRP) derived from platelets analyzed after treatment.

PDGFBB average values at baseline were higher in the PRP, these results are supported by those obtained by Passaretti F *et al.* in 2013, who compared the contribution of FCs by the PRP and PRF (Platelet-rich Fibrin), being the PRF (which is equivalent to this research to exudate), PDGF levels less than twice the PRP [37], possibly this happens because the PRP is platelet derived product where the highest concentration of these cells, and although not active Calcium submission to deep freeze made suffer platelets activation and subsequent thawing [38] to analyze the GF also caused complete lysis of them present in the PRP with the consequent secretion GFs total including PDGF. Unlike serum exudate is provided by the gel which was generated by activation with Calcium PRP; during both platelet gel formation as an undetermined concentration of GFs are trapped, thus remaining in the serum is a remnant of the same like a very limited number of platelets or low.

Now with regard to the levels of PDGF in the PPP before treatment, these are high when compared with those reported in the literature, however, this work was followed Anitua protocol [21] exactly one contemplated that centrifugation 0.5 cc of the first phase superficial plasma obtained after the procedure is considered PPP, and this was analyzed although it was confirmed that the average platelet count was $375.14 \pm 112.6 \times 10^3 \text{ x mm}^3$ and hence was not as poor as it is assumed in the Anitua's technique, by which it dismissed has been caused platelet activation during the preparation of the same and that this is responsible for the elevated levels of GF.

PDGFBB levels obtained for PRP, PPP and exudate baseline were compared with those found in other studies, and storing the differences as far as the method of obtaining the PRP and methodological framework reported work is concerned, it can be concluded that there are similarities and closeness between the results obtained here and those reported in other studies, such as Weibrich *et al.* (2002). These authors determined PDGFBB levels and other GFs in PRP from blood donors, obtaining average values of $10 \pm 8 \text{ ng / ml}$ to this factor [39-45].

The foregoing suggests that even though the same technique was applied to determine the FC (ELISA), the lack of standardization of the different commercial kits available makes it difficult to approval of the results even when based on the same principle [46].

PDGFBB average levels in PRP and its derivatives before and after the administration of Clopidogrel (Table 1) yielded statistically significant differences for PRP ($p < 0.0005$), PPP and exudate ($p < 0.0001$); with values lower than those found in these specimens after administration of aspirin. These results might be revealing a higher level of involvement of PDGF production capacity by platelet products treated subjects Clopidogrel. Was kept constant for higher concentrations of PDGFBB presented in PRP before and after treatment, and PDGFBB average levels before treatment were similar to those reported by other researchers whose values ranged from 2.3 to 37 ng / ml. Among these studies, it is worth mentioning that conducted by Christgau M *et al.* (2006) who assessed the levels of GF and cytokines in platelet concentrates from blood donors establishing its correlation with periodontal regeneration. In this study PDGFBB levels were at $15.8 \pm 7.9 \text{ ng / ml}$, considering such high levels [39, 40, 42, 44, 45].

Moreover, with respect to VEGF concentrations before and after treatment with ASA/Clopidogrel, it appears that there were no statistically significant differences between baseline levels GF and after treatment with ASA in any of the three PRP's derivatives.

On the contrary, when comparing baseline VEGF with those obtained following treatment with Clopidogrel was obtained statistically significant differences in the three derivatives (PPP: $p < 0.001$, PRP and exudate: $p < 0.005$). VEGF concentrations in the clopidogrel group were below baseline levels, in contrast to the values obtained in the group of ASA that were above basal levels but were not high enough to shed significant differences, what makes you think the Clopidogrel showed a higher level of involvement of VEGF concentrations compared with the ASA group.

VEGF levels obtained for PRP, PPP and exudate baseline were compared with those found in other studies, like that performed by Eppley B *et al.* in 2004, who quantified in healthy subjects undergoing plastic surgery the number of platelets and growth factors in platelet concentrate. Finding VEGF levels in a range of 155-955 pg / ml, these values being similar to those obtained in this work. Other authors include Castillo T *et al.* in 2011 and Durante C *et al.* in 2013, also obtained similar results to those reported in this work ($300-1200 \text{ pg / ml}$ and $933 \pm 120 \text{ pg / ml}$ respectively) [40,41,47].

It is noted that GFs levels (PDGF y VEGF) are higher in different platelet products AAS group, in

contrast to the clopidogrel group. What seems to reveal a less severely affected PDGFBB y VEGF levels in samples of subjects treated with ASA compared with clopidogrel group. It is inferred that these differences may be due in part to the specific pharmacological action mechanism that each drug has; in PRP, the ASA may completely block platelet aggregation induced by arachidonic acid (AA) and partially reduce platelet activation induced by collagen, ADP, epinephrine and platelet activating factor (PAF). Furthermore, thienopyridine among which is the Clopidogrel exert its primary function receptor blocking known as P2Y ADP, inhibit the secretion of the granules α which are rich in α GFs and interfere with platelet fibrinogen receptor (GIIb -IIIa), preventing further amplification pathway of platelet aggregation, which may have altered the secretion of VEGF and PDGF mainly Clopidogrel group [1, 48-50].

Importantly, in the literature reviewed, there were no scientific studies that had been conducted PDGFBB determinations after treatments used here, for which we could not establish any comparison with another similar experience. This observation unpublished results obtained in this investigation.

Furthermore, regarding PDGFBB levels obtained in the subjects studied, these were found to elevated levels before treatment and even experienced a decrease statistically significant in some cases after the administration of antiplatelet and particularly after Clopidogrel, this decrease was not pronounced unlike what was observed for VEGF, considered therefore that the use of a single dose of these drugs do not appear to affect significantly the secretion of PDGF by platelets of the subjects studied.

Although VEGF levels after administration of ASA were elevated when compared with baseline levels, however were not significant, which could have indicated that aspirin seemed not significantly affect the levels of this growth factor unlike Clopidogrel group, the levels of VEGF showed a significant decrease in relation to the baseline

Further work is needed to clarify whether prolonged treatment with these antiplatelet drugs in a larger population could markedly alter platelet count and GFs levels in PRP and its products, which may affect the use of autologous PRP in various medical treatments.

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CONFLICT OF INTEREST STATEMENT

It is stated that the authors of this manuscript do not have any conflict of interest.

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