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Controversial Issues on the Clinical Use of Platelet-Derived Growth Factors

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ABSTRACT

Increased levels of growth factors and cytokines are part of the physiological response to tissue damage. Platelet activation triggers repair mechanisms that take place consecutively in a coordinated and modulated way. Thus, the uncontrolled use of these tools, based on the principle that more is better, may lead to unexpected outcomes. For this reason, as suggested by most of the systematic reviews that address this issue, its clinical use should be carried out in randomized controlled trials, with strict standardization of protocols.

Keywords: Platelet-Rich Plasma; Controversy; Processing; Clinical Use; Growth Factors

Introduction

Platelet concentrates have been commonly used in clinical practice as blood components for hemotherapy treatments. Moreover, in the last decade of the past century, its clinical use for non-transfusion application was proposed [1,2]. Platelets contain dense granules and **α**-granules. These latter contain bioactive substances (as cytokines, chemokines and growth factors) which play a relevant role in wound healing phases. Updating the Celsius' quadrilateral (redness, swelling, heat, and pain) these phases are hemostasis, inflammation, proliferation, and remodeling [3,4]. They are developed as continuous and overlapping processes under strict coordination. The content of platelet granules has been commonly used to supplement basal culture media to stimulate *in vitro* cell proliferation, either as serum (after blood clotting) or platelet lysate (by cryofracture) [5].

Thus, it would be reasonable to consider the therapeutic use of platelet-derived bioactive substances for the treatment of injured tissues. Nevertheless, as an easily available resource, the expansion of its use has been characterized by a limited availability of large randomized controlled trials and evidence-based indications [6]. This paper is focused on addressing those issues that make comparative analysis of results difficult as well as those that remain controversial.

Terminology

The definition of the product is essential to establish a first level of standardization. To do this, five conditions of the final product should be considered [7,8,9]:

a) Raw material: Sample of whole blood (adult, cord blood) or platelet concentrate prepared by apheresis.

b) Processing system: Open or closed system using automated method. Number and parameters (relative centrifuge force and time) of spin cycles.

c) Platelet integrity: Platelet-rich plasma obtained by suspension of platelets in plasma after centrifugation of whole blood. Growth factor-rich plasma, when the granular content of platelets is released prior to clinical use, either by platelet activation or cryofracture (as platelet lysate, indicating temperature and number of freezing/thawing cycles).

d) Grade of platelet concentration: The efficiency of production is classified showing the recovery rate in platelets.

e) Presence of white blood cells: Depending on whether leukocytes are discarded during processing or not, the specification as poor or rich in leukocytes should be added.

f) Type of presentation: When coagulation is induced to facilitate its clinical use the term plasma can be changed by gel.

Whole Blood Processing

Donors profile must be known to evaluate potential interindividual variations. Even, differences in the volume of the blood sample can condition the yield of the proceeding [10]. For this reason, other variables which should be cited are type of anticoagulant (citrate, EDTA, if used), sample volume and detailed hematimetric values. The application of high values of relative centrifugal force during the separation of blood components can lead to platelet activation and, consequently, loss of released factors in the supernatant [11]. Cavallo et al. observed that the product obtained with a single centrifugation seemed to stimulate anabolic processes while that obtained with double centrifugation promoted more catabolic pathways [12].

Therapeutic Threshold Value

One of the most cited references in this regard was reported by Marx [13], according to which a concentration of 10^6pq/**µ**l is required to obtain a therapeutic effect, without yielding better results at higher concentrations. This assertion was later checked in an *in vitro* study [14]. However, in that study, 10% concentration was used as supplement of culture medium for mesenchymal cells. Thus, this concentration would correspond approximately to the basal one. Different authors have reported similar results from *in vitro* studies [15-23]. Some of these studies evidenced better results with the lower concentrations. Likewise, Boswell et al. [24] suggested a maximum biological threshold for platelet concentration to obtain anabolic upregulation.

Anticoagulant Agent

Citrate is well-known for maintaining the structural integrity and physiologic responsiveness of platelets [25,26]. Controversial results have been reported on the effect on platelet function for EDTA [26,27]. Regarding the efficiency in platelet count recovering, EDTA provided better yield than citrate [10,28,29].

Leukocytes

Some authors support the presence of leukocytes as beneficial, but with limits for their type, concentration and status of activation [7]. In addition to increasing the yield of growth factors, leukocytes can collaborate in the antimicrobial potential, as well as regulating inflammatory response and releasing mediators with analgesic effect. Conversely, others say that could increase inflammatory response [10,30].

Antimicrobial Potential

Platelets have been associated with the defense system by releasing microbicidal proteins and chemokines that share molecular structure with antimicrobial peptides (acquiring both chemotactic and microbicidal functions) [31-33]. Several authors have demonstrated this potential in deleukocyted platelet concentrates, resulting more efficient with activated platelets [34,35]. However, while the growth of some bacterial species is suppressed, in others the presence of platelets has a stimulating effect [36].

Platelet Activation and Clotting

Thrombin and calcium chloride are widely used to trigger clot formation [37,38]. But different processing systems generate different standards of structural quality in the fibrin matrix that is obtained with coagulation. Models to standardize the structure of fibrin networks have been proposed [39]. The use of thrombin for platelet activation has been associated to the inhibitory effect on cell differentiation [40]. When free zing/thawing cycles are used to platelet content releasing, the number of recommended cycles varies from 1-2 [41-43] to 3-5 cycles [44].

Platelet Count and Growth Factors Concentration

A correlation between platelet and growth factors concentrations has been observed for PRPs with significant differences in platelet concentration. However, it is more difficult to observe for shorter differences [45]. This could be the reason why there remains controversy over the relationship between these two parameters [46,47]. It seems that not all the factors are associated with this correlation, but there is no consensus on what they are [48,49]. The most significant case corresponds to vascular endothelial growth factor that is not detected by some authors [50] while its concentration is especially increased for others [37]. Differences in growth factor concentration have also been raised depending on the processing system used [51]. Part of the platelet content is released after activation in the form of micro vesicles and, therefore, it is not detectable by commonly used analysis systems [52]. These micro vesicles play an important role as cell signaling mechanisms [53-55]. The use of protocols based on double centrifugation yields a significative reduction in micro vesicles production [56].

Clinical Application

Although platelet-rich plasma is usually activated prior to infiltration, it is not clear whether this is necessary [57]. Dose (1-5

units) and interval between administrations (weekly, monthly) are other parameters in which differences among authors are also observed [58-60].

Study Design

In general, most studies correspond to case reports or retrospective clinical studies (sometimes without a control group), rather than prospective randomized clinical trials. Also, in some cases, the use of platelet rich plasma (PRP) is compared to placebo instead to the gold standard commonly used as alternative for that clinical indication. The International Cellular Medical Society has established a guide with recommendations aimed at standardizing issues related to the clinical use of PRP [61].

Conclusion

Platelet-rich plasma is a safe therapeutic alternative, but it is not a panacea for every pathological challenge involving tissue injury. In fact, it seems to yield better outcomes in acute pathologies than in chronic ones, and in younger patients. In Biology, more is not always better.

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