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COMMENTARY

Platelet-rich plasma therapy and reproductive medicine

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Abstract
Reports on clinical uses of platelet-rich plasma (PRP) have dramatically increased in the last decade. Indications for PRP therapy range from muscle and skeletal injuries to hair re-growth. More recently evidences have shown its positive effects in promoting endometrial and follicular growth and gestation in assisted reproduction cycles. We discuss the putative role of PRP on endometrial receptivity, with a brief history of its applications in research and clinical therapies. Despite its widespread uses in medicine, the mechanisms through which PRP exerts its regenerative effects are only postulated, not based on scientific data. There is an unmet need for advanced research to corroborate present findings in the clinical scenario.

Introduction

Platelet-rich plasma (PRP) is described as "a volume of plasma that has a platelet count above baseline" [1]. Platelets are small, anucleated cell fragments (2 to 3 μm in diameter) released from megakaryocytes found in the bone marrow. They contain numerous proteins, several growth factors (GFs) [2], and cytokines stored in cytoplasmic granules. The physiologic actions of some of the proteins have been studied, including GFs, peptide hormones, and chemoattractants for macrophages, neutrophils, stem cells, and several hundred other proteins, such as fibrinogen and fibrin. In addition, there are proteins with antibacterial and fungicidal actions. The dense granules in platelets contain adenoside diphosphate (ADP), adenoside triphosphate (ATP), calcium ions, histamine, serotonin, and dopamine, which represent important factors for tissue homeostasis.

Among the several growth factors stored and released by platelets, there are the platelet derived growth factor (PDGF), the epidermal growth factor (EGF), the insulin-like growth factor (IGF-I), the transforming growth factor b-I (TGFb-I), the vascular endothelial growth factor (VEGF), the hepatocyte growth factor (HGF), and the basic fibroblast growth factor (bFGF) [2]. The wide variety of elements found in platelets granules act synergistically, under normal physiological conditions, on local cells to promote wound healing.

Platelet granules undergo exocytosis, when an exogenous or native factor activates them. Example of an activating agent is the native collagen found in the extracellular matrix of nearly all tissues in human body.

After the first round of GFs release at activation, continued exocytosis maintains GF levels three- to fivefold higher as compared to baseline values [1].

PRP preparation methods and classification

Different techniques for PRP preparation can be found in literature. Each preparation method is intended to create an end product with a particular bioaction, and consequently, with a specific clinical application. Thus, PRP is not one single final blood derivate containing plasma and high concentrations of platelets. According to Dohan Ehrenfest and colleagues [3], platelet concentrates can be classified in four categories, depending on their leucocyte and fibrin content: pure platelet-rich plasma (P-PRP), leucocyte- and platelet-rich plasma (L-PRP), pure platelet-rich fibrin (P-PRF), and leucocyte and platelet-rich fibrin (L-PRF). In each category, the concentrate can be produced by different processes.

Most of the described PRP preparation methods involve similar procedures, such as blood collection in presence of an anticoagulant and immediate centrifugation.

The short, mild-spin centrifugation aims to separate the whole blood into three layers: the supernatant corresponding to the acellular plasma, the intermediate "buffy coat"

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containing the concentrated platelets and last, the bottom pellet rich in red blood cells. After the first centrifugation, a second faster and more prolonged spin may follow, to further isolate the "buffy coat". Finally, at administration on the surgical or wound site, an *activating factor*, such as thrombin may be added to the final platelet concentrate to promote platelet degranulation and exocytosis of the factors stored in the cytoplasmic granules.

Variability can be seen in the total platelet count from one patient to another and the overall goal is to achieve a concentrating factor of two to three times in whole blood for any given patient [4].

A simpler methodology described in literature eliminates both, the anticoagulant agent for blood collection and the activating factor used for platelet activation [5]. In this situation, a leucocyte- and platelet-rich fibrin (L-PRF) clot can be collected after a single centrifugation step and applied directly to wounds. However, when no anticoagulant is used, platelets can be activated by the mechanical stress of centrifugation [4].

Clinical applications of PRP

The pioneering work of Danielli [6], who systematically analyzed the role played by platelets in vascular integrity in vitro, paved the way for research on the use of whole blood for organ perfusion. Similarly, Folkman and coworkers stated the importance of platelets in the maintenance of vascular integrity, when they suggested that tumors did not grow beyond a certain size, because they failed to grow new endothelial cells [7]. Using the dog model, the same group of scientists showed that perfusion of thyroid gland and kidney in vitro with platelet-rich medium presented an improved vascular preservation and tissue survival [8]. In the words of the authors "….platelets seem to 'nurture' the microcirculation in some way." As the authors hypothesized, these observations had profound implications for short- and long-term preservation of organs.

In regenerative medicine research, multipotent mesenchymal stem cells obtained from deciduous teeth or umbilical cord were successfully maintained in culture and retained their differentiation capacity, when xenogenic fetal calf serum was replaced by autologous PRP [9, 10]. These reports represent important steps towards the generation of clinical grade stem cell lineages for human use, free from the presence of animal-derived products during isolation and culture.

Further, PRP can be considered as an autologous source for tissue engineering applications [11]. The use of PRP as scaffold for the intracerebral administration of bone marrow stem cells resulted in a significant neurologic improvement in experimental animals presenting induced intracerebral hemorrhage. In addition, post-mortem histologic analysis showed that animals that received PRP scaffolds had the stem cells integrated in the injured tissues and presented endogenous neurogenesis, measured by the expression of specific glial proteins and neuronal nucleus [12].

PRP and human reproduction

The first trial on the use of PRP in human reproduction technologies was reported by a Chinese group [13] to improve endometrial thickness in patients undergoing IVF treatment. Five patients whose infertility treatment could not be accomplished due to poor endometrium growth in previous cycles were treated with PRP prepared from autologous blood. PRP infusion was administered directly into the uterine cavity on the 10th day of hormonal replacement therapy, immediately after preparation. Except for one patient whose gestation ended in an abortion of a XO fetus, the other four went successfully to term.

Since the first IVF attempts in the mid-1970s, researchers are aware of the important role played by the endometrium, not only by the embryo itself, in achieving a pregnancy. After nearly four decades of research and clinical trials, Assisted Reproduction Technologies (ART) have significantly improved pregnancy and birth rates, as new methodologies allowed embryonic growth to the blastocyst stage and genetic screening helped to choose "the best embryo" for transfer. Endometrial receptivity, however, remained an unsolved problem. In a recent comprehensive review on refractory endometrium, Garcia-Velasco and colleagues [14] concluded that there is not available evidence to help physicians and patients on how to improve a poor endometrium.

A similar report was recently published on the use of PRP to improve endometrial thickness in a series of 10 patients with history of frozen-thawed embryo transfer (FET) cancelations due to poor endometrial growth [15]. All 10 patients presented an endometrial thickness above 7 mm after PRP administration and five became pregnant after FET. One gestation ended in a miscarriage and four are ongoing pregnancies. The authors concluded that PRP was effective in inducing endometrial development in patients with thin, poor endometrium. Despite the encouraging results, it is difficult to draw a single conclusion from these two reports, as one study showed the comparison of endometrial growth between different cycles (cycles without PRP and cycle with PRP administration [13]) and the second study showed the effect in the same cycle, as PRP was gradually administered [15]. In both situations, endometrial thickness improved for all patients to or above 7 mm. However, some patients received one [13] or two PRP infusions [13, 15], and progesterone support was started when, endometrium reached the adequate thickness for embryo transfer [15].

Thus, it is not clear how the intrauterine administration of PRP may act to affect endometrial thickness. Results from studies on the role of endometrial thickness on implantation and live births are contradictory. While some authors reported that there is not a relationship [16, 17], there is one retrospective study that shows that there is a steady and gradual increase in pregnancy rates as endometrial thickness increases [18]. Furthermore, the last authors claimed that this relationship exists independent of maternal age and embryo quality.

Considering that cell proliferation and tissue growth depend not only on single growth factors, but on an overall favorable environment, another possible explanation on the mode of action of PRP on endometrial growth and receptivity is via its anti-microbial, anti-inflammatory properties, as demonstrated on human chondrocytes [19] and on equine and bovine uterine infections [20–23]. In humans, chronic endometritis (CE) has a high prevalence among infertile patients (range 2.8 to 46%) [24, 25]. CE may be caused by a variety of infectious agents such as bacteria, viruses, and parasites [26]. Generally, endometritis is asymptomatic or only accompanied by mild disturbances. Even histological analysis of endometrial biopsies may miss the diagnosis, as the identification of leukocytes in the endometrial stroma is a normal finding, particularly before menstruation [27, 28]. In ART, the presence of endometritis is associated with overall lower success rates and 30.3% of the patients with repeated implantation failure are diagnosed with CE [28, 29].

Despite these evidences, several clinicians consider that CE does not to affect the reproductive status and general health of affected women [30]. However, a recent study showed an association between aberrant decidualization in vitro and the presence of CE in infertile patients [31]. The authors observed that the expression of prolactin and insulin-like growth factor binding protein-1, markers of decidualization, and of steroid hormones receptors were altered in endometrial samples from CE patients. These results may explain the effects of CE on implantation and maintenance of pregnancy previously reported in ART cycles [28, 29].

Considering these evidences, it seems plausible to suggest that the positive effects on implantation and gestation observed in the two reported clinical trials of PRP on ART occurred via its anti-inflammatory molecules on an undetected (silent) endometritis. However, one cannot dismiss the important role played by platelets growth factors on cell proliferation and neo-endothelial cell generation, key elements for an appropriate endometrial receptivity.

In the field of fertility preservation for women with cancer, a recent study [32] demonstrated that in vitro follicular growth from primordial or primary to pre-antral stage is enhanced by the addition of PRP to the culture medium. Isolated primordial or primary follicles from fresh or cryopreserved ovarian tissue cultured for 10 days in presence of PRP showed a significant greater growth rate and cell viability than follicles cultured in medium supplemented with fetal bovine serum. Also, the PRP in autografts of cryopreserved human ovarian tissue may have contributed to the successful pregnancy and birth after the first stimulation cycle in an oophorectomized patient, with undetectable level of AMH [33]. These last reports [32, 33] emphasize the putative role of PRP growth factors on cell proliferation and neoangiogenesis promoting follicular survival and development.

Conclusion

Despite the increased use of PRP in musculoskeletal injuries, dentistry, and other medical fields including human-assisted reproduction, the need of standardization of PRP preparation methods for clinical use is evident [34]. Also, data from clinical studies are limited, majority are not randomized trials. Thus, there is the urge for well-designed randomized studies and basic research on the cellular and molecular level to improve our knowledge on PRP *mode of action* to better understand on how and in what clinical situations it should be administered.

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