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Platelet-rich plasma activation: are there differential effects in reproductive medicine?

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Abstract

The aim of *this in vitro* short report was to determine whether activation of platelet-rich plasma (PRP) results in differential effects on growth factor release and cell proliferation in reproductive medicine. This study involved PRP from five donors, activated with either $CaCl_2$ (exogenous activation) or type I collagen (mimicking endogenous activation). The release of growth factors (EGF and PDGF-AB) and the proliferative response of human ovarian fibroblasts were analysed. PRP activated with $CaCl_2$ formed stable clots and released statistically significant higher levels of EGF (871 ± 426 pg/mL) and PDGF-AB (26535 ± 6477 pg/mL) compared to collagen-activated PRP (EGF: 141 ± 66 pg/mL, PDGF-AB: 13060 ± 2301 pg/mL). Additionally, $CaCl_2$-activated PRP induced greater proliferation in ovarian fibroblasts (34.90 ± 17.82 ng/mL DNA) than collagen-activated PRP (30.75 ± 18.21 ng/mL DNA). In conclusion, the activation of PRP with $CaCl_2$ results in higher growth factor release and a stronger biological response compared to type I collagen-activation. These findings underscore the importance of standardized PRP activation protocols to enhance clinical outcomes in reproductive medicine.

Keywords Assisted reproductive techniques, Growth factors, Platelet-rich plasma, PRGF, PRP, Regenerative medicine

Introduction

The use of platelet-rich plasma (PRP) is becoming increasingly prevalent in various medical fields. Its application in assisted reproductive techniques (ART) is witnessing the most rapid growth [1] due its capacity to stimulate angiogenesis and promote cell proliferation [2]. Indeed, the role of platelets has been extensively described at all stages of reproductive physiology, including folliculogenesis, ovulation, placental development, implantation and embryogenesis [3]. PRP is a promising treatment for infertility with potential implications in the field of reproductive medicine. This therapy promotes endometrial thickening, favours embryo implantation, participates in ovarian reactivation, improves oocyte and embryo quality, and supports the viability and growth of early human preantral follicles [4–10].However, there are conflicting clinical results, which may be partially explained by the variability in the protocols used to obtain and apply PRP [11]. One of the key issues responsible for the variability in reported clinical outcomes is the activation of PRP. Controlled activation of platelets and the coagulation cascade can be achieved with Ca^{+2}, mainly $CaCl_2$ (exogenous activation). Nevertheless, some protocols rely on activation of the PRP in the target tissue itself and do not activate, leaving activation mainly to tissue collagen (endogenous activation). It should be noted that most PRP collection systems anticoagulate the blood with citrate, which chelate Ca^{+2} in the blood and prevent clotting and platelet activation. To shed light on the importance of PRP activation, PRP from five donors

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was activated with $CaCl_2$ and type I collagen. The release of growth factors and the proliferative response of ovarian fibroblasts were then examined.

Methods

The study protocol was approved on November 7, 2022, by the Araba University Hospital Clinical Research Ethics Committee (BTI-01-IV/22/CPL). No registration was required for this in vitro study (Clinical Trial Number: not applicable).

Preparation of PRP

Figure 1 shows the PRP preparation scheme and methodology. PRP was prepared according to the PRGF-Endoret (Plasma Rich in Growth Factors) technology (KMU 19, BTI Biotechnology Institute, Vitoria, Spain). Blood from five healthy donors (3 females and 2 males; mean age 47.8 ± 16.9 years old) was withdrawn into 9-mL tubes containing 400 µL sodium citrate (3.8%) as an anticoagulant. The tubes were centrifuged at 580 g for 8 min at room temperature (RT) in a System V centrifuge. The non-activated liquid PRGF was obtained by fractioning the whole plasma column, excluding the buffy coat. A haematological analysis (Pentra ES 60, Horiba ABX SAS, Montpelier, France) was performed to characterize the whole blood and the liquid PRGF prior to activation.

PRP activation

Plasma from each donor was distributed into two separate aliquots: the first one was activated with 20 µL of 10% $CaCl_2$ (PRGF activator) per mL of PRGF and the second aliquot was activated with 11.1 µg collagen I (Hart Biologicals, Hartlepool, UK) per mL of PRGF (Fig. 1).

Samples were examined after one hour at 37 °C for clotting and retraction, and centrifugated at 1000 g for 10 min at RT. Then, the clots were removed and the PRGF supernatants were filtered through 0.22 µm PES filters, aliquoted, and stored at -80 °C until use.

Growth factor content

Epidermal growth factor (EGF) and platelet-derived growth factor (PDGF-AB) were quantified in duplicate by ELISA (R&D Systems Inc-Bio-Techne, Minneapolis, USA) in $CaCl_2$- and collagen-activated samples. Results were expressed as pg/mL.

Cell proliferation assay

Human ovarian fibroblasts (HOF, Innoprot, Derio, Spain) were seeded at a density of 5000 cells/cm^2 in their routine culture medium in 96-well dark-bottom plates for 24 h. After removing the medium, the cells were cultured with basal medium supplemented with 10% of plasma supernatant, either $CaCl_2$- or collagen- activated for 72 h. Cell proliferation was quantified by the change in DNA content (Cyquant assay, Thermo Fisher, Waltham, USA).

Statistical analysis

Results were expressed as mean ± standard deviation. Normality was tested using the Shapiro–Wilk test. Paired-sample t-test was used to compare both groups. Statistical analyses were performed with SPSS Statistics 15.0 (IBM, Chicago, USA) with a significance level of $p < 0.05$.

Results

PRP characterization

The mean platelet concentration (x10^3/µL) in peripheral blood and PRGF was 255 ± 37 and 486 ± 95 respectively, which is a 1.9 ± 0.2-fold increase over the peripheral blood value. Platelet recovery in PRGF was 85 ± 3.8%.

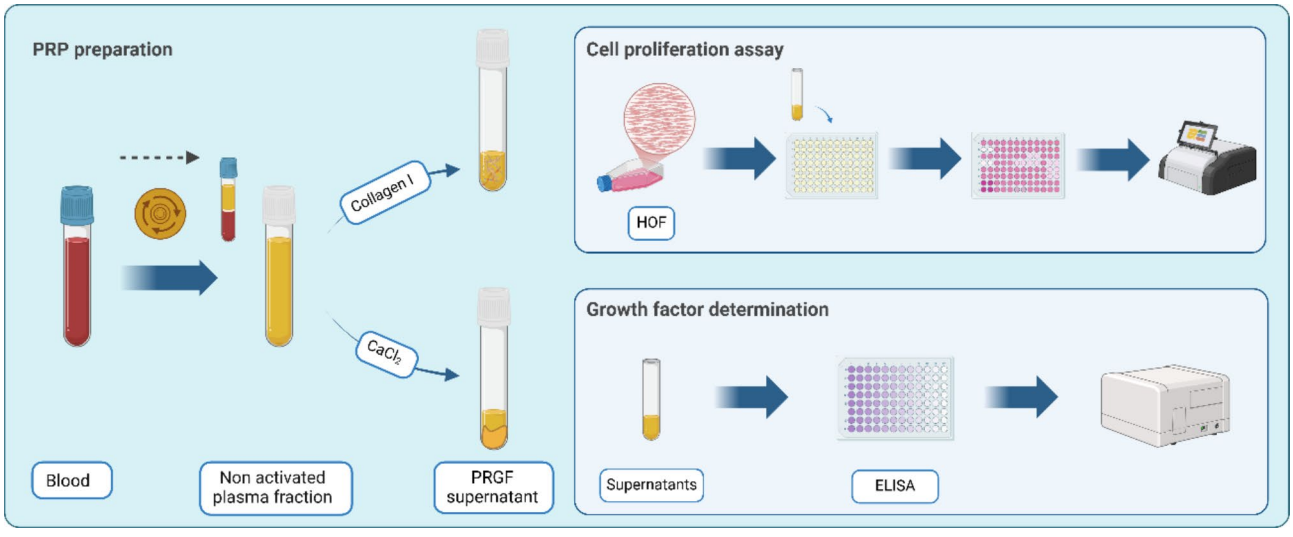

Fig. 1 Scheme of platelet-rich plasma (PRP) preparation and layout of experiments, including cell proliferation and ELISA assays

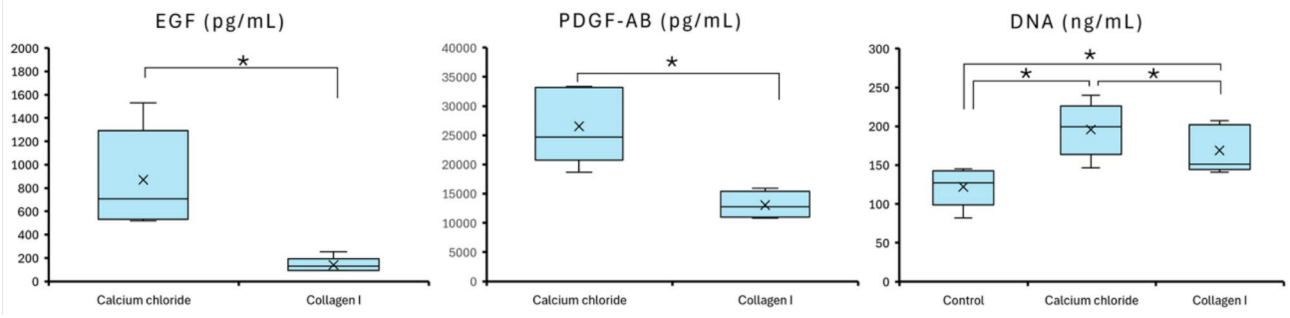

Fig. 2 EGF and PDGF-AB levels (pg/mL) in the $CaCl_2$ and collagen I activated samples and cell proliferation of human ovarian fibroblasts (measured as DNA in ng/mL) after 72 h incubation with both preparations (* $p < 0.005$)

Leukocyte mean concentration ($x10^3/\mu L$) in peripheral blood was 6.4 ± 0.5, whereas in PRGF it was almost zero (0.2 ± 0.1). The mean value of erythrocytes ($x10^6/\mu L$) in peripheral blood was 4.80 ± 0.42 while in PRGF was almost undetectable (0.01 ± 0.01).

Clot formation

Activation with $CaCl_2$ resulted in the formation of a firm and stable clot, which exhibited retraction after one hour of incubation at 37 ºC for all donors. Conversely, incubation with type I collagen did not result in clot formation, but rather the formation of a gelatinous mass with no apparent clot retraction.

Growth factor content

The release of two platelet growth factors exhibited notable differences between the two activation modes (Fig. 2). EGF was 871 ± 426 pg/mL in $CaCl_2$ activated samples and 141 ± 66 pg/mL in collagen activated samples ($p < 0.005$). For PDGF-AB, higher levels were also observed in $CaCl_2$-activated samples compared to collagen-activated samples, 26,535 ± 6477 versus 13,060 ± 2301 ($p < 0.005$).

Cell proliferation

HOF cells exhibited a statistically significant increase ($p < 0.005$) in proliferation when the culture medium was supplemented with $CaCl_2$-activated plasma (34.90 ± 17.82 ng DNA/mL) compared to the collagen I activated group (30.75 ± 18.21 ng/mL) (Fig. 2). In addition, the proliferative response exhibited by both groups was statistically significantly higher than that of the control group (supplier's recommended medium).

Discussion

The main findings of this study are that the activation with $CaCl_2$ produces more platelet growth factors and a greater biological response than collagen I activation. Our results are in line with those recently reported [2] regarding the need to activate the applied PRP; these authors speculate that activation may not be necessary because the resting platelet membrane receptors (CD40 and $\alpha IIb\beta_3$) may be sufficient to stimulate folliculogenesis, but on the other hand they point out the importance of the molecules released during platelet degranulation. Nevertheless, our findings are also consistent with those of Cavallo et al. [12], who demonstrated that the release of PDGF, TGF-β, and VEGF in $CaCl_2$-activated PRP samples were superior to those in PRP activated with collagen. Based on these findings, the researchers concluded that collagen I could be classified as a weak platelet activator. Similarly, Rickers et al. propose exogenous activation of PRP rather than infiltration of non-activated PRP to optimize ovarian follicular response, suggesting that PRP-derived molecules have the potential to stimulate stem cell differentiation and proliferation by enhancing angiogenesis and, ultimately, modulating inflammation [13]. The importance of PRGF-containing growth factors in reproductive biology has been extensively described. Thus, EGF has been implicated in both ovulation [14] and oocyte development and maturation [15]. On the other hand, PDGF has been reported to promote the transition from primordial to primary follicles [16].

Recently, Serdarogullari et al. [17] discussed PRP preparation and activation protocols. However, in most of the 34 trials reviewed, there was no information on the activation. Regardless, all data from both PRP preparation and application should be reported to ensure reproducibility. In reproductive medicine, as in other areas, the full activation of PRPs allows for the whole platelet content that cannot be achieved without this step.

Our study has several limitations, including being preliminary, using a single cell phenotype, and the lack of clinical efficacy data. Despite this, further research is needed to elucidate the molecular mechanisms underlying the clinical efficacy of PRP and to determine the impact of PRP activation on the clinical outcome. In any case, PRGF appears to be a promising autologous adjuvant therapy for infertility, that is readily available and free of immunological reactions.

Abbreviations

ART	Assisted reproductive techniques
$CaCl_2$	Calcium chloride

EGF	Epidermal growth factor
HOF	Human ovarian fibroblasts
PDGF-AB	Platelet derived growth factor AB
PRGF	Plasma rich in growth factors
PRP	Platelet-rich plasma
RT	Room temperature
TGF-β1	Transforming growth factor-beta 1
VEGF	Vascular endothelial growth factor

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Author contributions
All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by E.A., M.d.l.F., R.P. and M.H.A. The first draft of the manuscript was written by M.d.l.F. and R.P., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. All named authors meet the International Committee of Medical Journal Editors (ICMJE) criteria for authorship for this article, take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.

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Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The protocol for this in vitro study was approved on November 7, 2022, by the Araba University Hospital Clinical Research Ethics Committee (BTI-01-IV/22/CPL).

Consent for publication
N/A.

Competing interests
The authors declare that E.A. is the Scientific Director of, and M.d.l.F., R.P. and M.H.A. are scientists at BTI-Biotechnology Institute I MAS D, a biomedical company that investigates the fields of regenerative medicine and PRGF-Endoret technology.

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