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SHORT REPORT

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Growth differentiation factor 11 (GDF11) – a promising anti-ageing factor – is highly concentrated in platelets

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Recent research suggests that growth differentiation factor 11 (GDF11) could reverse age-related diseases and that its blood concentration decreases with age. This poses plasma from young donors as a therapeutic GDF11 source to treat age-related diseases. In addition, the tissue source of circulating GDF11 remains unknown. We analysed GDF11 levels in paired samples of serum, plasma and platelet lysate (PL) from 23 volunteers. Plasma and PL were collected by plateletpheresis. Here, we show that GDF11 is highly concentrated in platelets and that the circulating levels reported in previous studies could be biased as a result of serum sample manipulation.

Key words: apheresis, cytokine, plasma, Platelet Rich Plasma (PRP), regenerative medicine ageing.

Introduction

Ageing is a complex process characterized by progressive loss of cellular fitness as a consequence of accumulated cellular damage [1]. Recent studies suggest that blood from young donors could reverse age-related diseases. This hypothesis is based on experiments, which show that blood from young mice could rejuvenate aged tissues in older ones [2–4]. After analysing several circulating factors, Loffredo *et al*. [3] suggested that GDF11 could be responsible for these effects. These studies suggested a new paradigm in the treatment of aged-related diseases, where blood from young donors could be used as a GDF11-rich source to reverse some aged-related diseases. On the other hand, new studies have undermined this hypothesis by indicating that GDF11 concentration in serum does not decrease with age but, conversely, increases [5]. In addition to this debate, the tissue source of GDF11 found in circulating blood remains unknown [6]. We hypothesized that GDF11 could be stored in circulating platelets and also that serum may contain higher concentrations than plasma as a result of sample manipulation errors. The aim of this study was to test these hypotheses by comparing GDF11 content in three blood products: serum, platelet-poor plasma (PPP) and platelet lysate (PL) obtained from the same volunteers.

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Materials and methods

Platelet Rich Plasma (PRP) and PPP samples were collected by apheresis from 23 volunteers as previously described [7]. Whole blood was also collected in EDTA and dry tubes for cell counting and serum separation. Briefly, all the samples were maintained overnight at 20°C to allow platelet disaggregation in PRP, and then, cell counting was performed. Samples underwent a double freeze–thaw cycle to disrupt cell membranes to allow cell content release. PRP is converted to platelet lysate during this process. The levels of GDF11 and PDGF-ββ were measured using an ELISA kit (Cusabio). Finally, the content of both cytokines was compared in PPP, serum and PL samples using Friedman's, ANOVA and paired t-

tests. In addition, multivariate regression analyses were performed to search for any relationship between GDF11 concentrations in samples and other variables, such as volunteer age. The study was approved by the Hospital Research Ethics Committee.

Results

Median (range) volunteer age was 39·3 (18·5–85·8) years. PRP platelet concentration was 1476·3 (95% CI: 1361·4–1591·1) $\times$ 10^9/l. Leucocyte and red blood cell counts in PRP were virtually zero. Mean GDF11 concentrations were 331 (95% CI: 248·1–413·9) pg/ml in platelet lysate, 23·30 (95% CI: 21·3–25·3) pg/ml in plasma and 34·30 (95% CI: 29·3–39·3) pg/ml in serum. Mean PDGF-ββ concentrations were 375·6 (95% CI: 352·8–398·4) pg/ml in platelet lysate, 127·2 (95% CI: 116·3–118·1) pg/ml in plasma and 27·3 (95% CI: 15·6–39) pg/ml in serum.

Mean GDF11 concentration in PL was 10·2 (95% CI: 7·6–12·9) and 15·0 (95% CI: 10·4–19·7) times higher than in serum and PPP, respectively ($p < 0·0001$ in both analyses). Mean GDF11 concentration in serum was 1·5 (95% CI: 1·3–1·8) times higher than in plasma ($p < 0·0001$) (Fig. 1). The multivariate analysis showed that older individuals had significantly higher levels of GDF11 in serum ($p = 0·004$) (see Fig. 2a). This relationship with age was not observed in plasma samples.

Discussion

This study shows that GDF11 is at least ten times more concentrated in platelet lysate than in serum or plasma, indicating that GDF11 is stored in platelets. Very low levels of GDF11 were detected in serum and plasma, which agrees with a previous report and could indicate that circulating GDF11 may have little physiological relevance [8]. In addition, we observed that GDF11 was more concentrated in serum than in plasma, suggesting that GDF11 could be released from platelets as described for other platelet-contained factors [9].

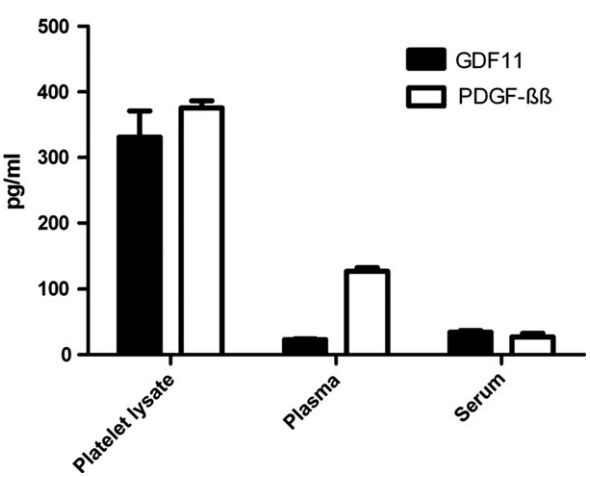

Fig. 1 GDF11 and PDGF-ββ concentrations in platelet lysate (PL), serum and plasma (PPP). The graphs show GDF11 and PDGF-ββ concentrations measured by ELISA. PL contained significantly more GDF11 than serum or plasma ($P < 0·0001$ in both analyses). Data are displayed as mean ± SEM.

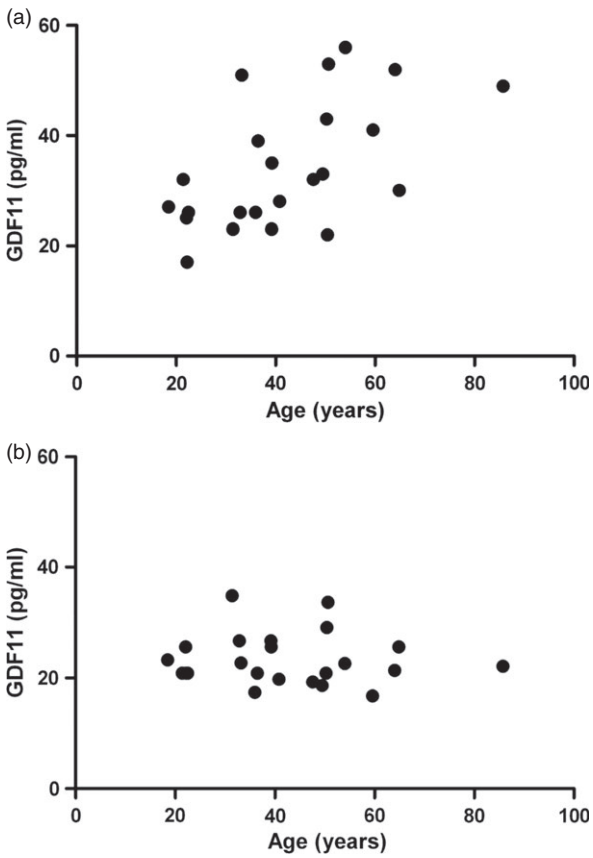

Fig. 2 Relationship between GDF11 levels and age. GDF11 levels increased with age in serum (a) ($P = 0·004$); but not in plasma (b).

No relationship was found between GDF11 levels in plasma and age. Interestingly, when serum was analysed, we found that GDF11 levels increased with age. This agrees with a previous report [5] and may be explained by the fact that platelet activation is enhanced in older humans [10], which would lead to greater release of factors during the clotting process *'in tube'* to obtain serum.

We used PDGF-ββ as a control, as it is a well-known intraplatelet protein, and we expected a higher concentration in serum than in plasma, as observed for GDF11. However, PDGF-ββ concentrations in serum were lower. This may be explained by the presence of specific binding proteins that may increase PDGF-ββ clearance as has been previously described [11].

The main strength of our study is that plateletpheresis was used to obtain a product enriched in platelets with virtually no leucocytes or red cells, and this allows us to infer that GDF11 is stored in platelets. On the other hand, PPP collection through an apheresis machine provided a virtually acellular plasma product, which is better for evaluating proteins circulating in human blood. We are also aware that our study has weaknesses. Our GDF11 concentration results require confirmation using other or more sensitive biochemical methods in a larger cohort of volunteers and also to explore if GDF11 could also be present in other blood cells that were not analysed in this study.

In conclusion, our results suggest that GDF11 is highly concentrated in platelets, which allows us to infer that, if GDF11 really possesses an anti-ageing or any other biological effect, platelets obtained from volunteers of any age should be used as a biological source of GDF11 instead of, or as an alternative to, serum (or plasma) from young people [12]. On the other hand, we believe that GDF11 levels in serum are not representative of the GDF11 that is really circulating in human blood. Therefore, further studies should be performed that focus on detecting circulating GDF11 in plasma rather than serum.

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Author contributions

JLB involved in conceptualization; JLB and MY involved in methodology; JLB, MY, CdM, and RMG involved in investigation; JLB and MY involved in writing of the original draft; JLB, MY, CV, CdM, RMG and JAGM involved in writing, review and editing of the manuscript; RC, CV, CR, AR and EF involved in the collecting the resources RC provided funding support; and JLB and RC involved in supervision and project administration.

Sources of funding

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Disclosures

Dr. Bueno is the founder and Medical Director of the company registered as 'PRoPosit Bio SL'. The other authors do not declare any conflict of interest.

References

1 López-Otín C, Blasco MA, Partridge L, *et al.*: The hallmarks of aging. *Cell* 2013; **153**:1194–1217

2 Conboy IM, Conboy MJ, Wagers AJ, *et al.*: Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature* 2005; **433**:760–764

3 Loffredo FS, Steinhauser ML, Jay SM, *et al.*: Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. *Cell* 2013; **15**:828–839

4 Sinha M, Jang YC, Oh J, *et al.*: Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. *Science* 2014; **344**: 649–652

5 Egerman MA, Cadena SM, Gilbert JA, *et al.*: GDF11 increases with age and inhibits skeletal muscle regeneration. *Cell Metab* 2015; **22**:164–174

6 Patel VK, Demontis F: GDF11/myostatin and aging. *Aging* 2014; **6**:351–352

7 Bueno JL, García F, Castro E, *et al.*: A randomized crossover trial comparing three plateletpheresis machines. *Transfusion* 2005; **45**:1373–1381

8 Rodgers BD, Eldridge JA: Reduced circulating GDF11 is unlikely responsible for age-dependent changes in mouse heart, muscle, and brain. *Endocrinology* 2015; **156**:3885–3888

9 Ahamed J, Burg N, Yoshinaga K, *et al.*: In vitro and in vivo evidence for shear-induced activation of latent transforming growth factor-beta1. *Blood* 2008; **112**:3650–3660

10 Mohebali D, Kaplan D, Carlisle M, *et al.*: Alterations in platelet function during aging: clinical correlations with thromboinflammatory disease in older adults. *J Am Geriatr Soc* 2014; **62**:529–535

11 Raines EW, Bowen-Pope DF, Ross R: Platelet-Derived Growth factor; in: Roberts A, Sporn M (eds): *Peptide Growth Factors and Their Receptors I.* Berlin Heidelberg, Springer-Verlag, 1990:21–22.

12 The PLasma for Alzheimer SymptoM Amelioration (PLASMA) Study - Full Text View - ClinicalTrials.gov