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Comparison of growth factor and interleukin content of adult peripheral blood and cord blood serum eye drops for cornea and ocular surface diseases

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ABSTRACT

Introduction: Various blood-derived products have been proposed for the topical treatment of ocular surface diseases. The aim of the study was to compare the different content of Growth Factors (GFs) and Interleukins (ILs) in peripheral blood (PB-S) and Cord Blood (CB-S) sera.

Materials and methods: Sera were obtained from 105 healthy adult donors (PB-S) and 107 umbilical/placental veins at the time of delivery (CB-S). The levels of epithelial-GF (EGF), fibroblast-GF (FGF), platelet-derived-GF (PDGF), insulin-GF (IGF), transforming-GF alpha (TGF-α,) and beta 1-2-3 (TGF-β1-β2-β3), vascular endothelial-GF (VEGF), nerve-GF (NGF), Interleukin (IL)-1β,IL-4,IL-6,IL-10, and IL-13 were assessed by Bio-Plex Protein Array System (Bio-Rad Laboratories, CA, USA). The Mann-Whitney test for unpaired data was applied to compare GFs and ILs levels in the two sources. The associations among each GF/IL level and the obstetric data for CB-S and hematological characteristics for PB-S were also investigated.

Results: The levels of EGF, TGF-α, TGF-β2, FGF, PDGF, VEGF, NGF, IL-1B, IL-4, IL-6, IL-10, and IL-13 were significantly higher in CB-S compared to PB-S. Conversely, the levels of IGF-1, IGF-2, and TGF-β1 were significantly higher in PB-S. The female sex and the weight of the child showed a significant association in predicting EGF and PDGF levels.

Conclusion: A significantly different content in those GFs and ILs was demonstrated in the two blood sources. Since each GF/IL selectively regulates different cellular processes involved in corneal healing, the use of PB-S or CB-S should be chosen on the basis of the cellular mechanism to be promoted in each clinical case.

1. Introduction

Blood derived products for the treatment of ocular surface diseases have been introduced some decades ago [1], and became increasingly popular in recent years [2]. Blood-derived products contain growth factors (GFs), cytokines, vitamins and nutrients present in natural tears, and aiming at promoting epithelial cell homeostasis, growth and migration. Therefore, the rationale for their use is to supply tears with these compounds.

Several different blood derivatives have been proposed for the treatment of various ocular surface disorders, in particular corneal wound healing (CWH) and dry eye disease (DED). Blood-derived products include eye drops prepared either from patients' own peripheral blood serum (autologous serum, AS) [2–4], platelet rich plasma [5–8], plasma rich in GFs [5] and platelet lysate [9,10], or from donors, such as allogeneic umbilical cord blood serum (CB-S) [11–15] and allogeneic serum [16,17].

To date, no consensus has been reached about the methods of preparation, the quality control assessment, and the posology and duration of treatment [3,4,18].

In the absence of standardization, it is difficult to compare the levels of epitheliotrophic substances in the final products [19], as well as their therapeutic efficacy. In addition, only few prospective, randomized and controlled clinical trials comparing these products have been performed, and a recent Cochrane meta-analysis questioned the efficacy of autologous serum, the most popular among overall blood derived products [20].

The purpose of this work was to analyze and compare the content of a wide panel of epitheliotrophic growth factors and cytokines in PB-S from healthy donors versus CB-S, two allogeneic sources that can be utilized for the treatment of severe keratopathy.

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2. Materials and methods

This is a prospective study on blood serum samples obtained from the Emilia Romagna Cord Blood Bank and from the Transfusional Service of the S.Orsola- Malpighi Teaching Hospital. The study was performed in respect to the principles in the Declaration of Helsinki. The study was approved by the local Ethical Committee (number of protocol 100/2016/O/Sper). Donors were requested to sign an informed consent after having been explained the scientific rationale of the study. Blood samples were collected from 105 donors by venipuncture at the time of donation and 107 cord blood samples at the time of delivery.

2.1. Obstetric data

Data from the following obstetric factors were retrieved in anonymous from clinical records: parity and gestational age of the mother, sex, birth weight and Apgar score of the newborn, placental weight, duration of labor and the mode of delivery.

2.2. Blood collection method

CB units had been collected after a donor selection questionnaire based on international criteria for cord blood banking. CB was collected from spontaneous term births free of complications (≥37th week of pregnancy) and Caesarean births, by trained and qualified health personnel. All steps from the recruitment to the processing and registration of CB were performed according to guidelines edited by the Foundation for the accreditation of cellular therapy (FACT). CB collection for transplantation purposes was performed when the placenta was still in utero by puncturing the umbilical vein with a sterile system (Cord blood collection set, JMS, Singapore) and, for ophthalmic purpose, samples from ex utero placenta vessels were transferred in 9 ml Vacumtube (Biomed Device, Modena, Italy) without any anticoagulant. The medium volume of collected samples was 7 ± 1,5 ml.

Adults' PB was collected in 9 ml Vacumtube (Biomed Device, Modena, Italy) without any anticoagulant during normal donation. Adult' PB collected samples had always 9 ml volume.

For further processing, the unit and the related samples were sent to the Processing Facility (PF) laboratory opened 24/24 h, 7/7 days.

2.3. Assessment of CB unit

CB unit was taken to the PF of the CB Bank where it went through a series of checks and tests to establish the blood characteristics and its suitability for preservation and therapeutic use. Maternal infectious disease markers (HIV, HCV, HBV *Treponema pallidum*, CMV, Toxoplasmosis and HTLVI/II) evaluations were performed.

Collected adult' PB were tested for infectious disease markers of HIV, HCV, HBV and Treponema Pallidum according to italian regulations.

For both samples the hematological characteristics were assessed and in particular the number of white blood cells (WBCs), total nucleated cells (TNCs) and platelets (PLTs) were counted with an auto analyzer (XN-1000 Sysmex Europe GmbH, Germany) and expressed as number x 106 x ml. The number of CD34+ cells was measured by flow cytometry (Navios – Beckman Coulter, CA, USA) with a single-platform technique (Stem Kit, Beckman Coulter, CA, USA).

2.4. Blood serum samples

The test-tubes of peripheral blood were centrifuged at 2.800 g for 10 min and serum samples were transferred in sterile tubes under laminar flow hood and stored at −80 °C. Both cord and adult serum samples had an average volume of 4 ± 0.5 ml.

2.5. Growth factor and interleukin dosage

Serum from both sources were tested for the presence of growth factors: IL-1β, IL-4, IL-6, IL-10, IL-13, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), nerve growth factor (NGF), epidermal growth factor (EGF), transforming growth factor (TGF)-α, TGF-β1/β2/β3, insulin growth factor (IGF)-1 and IGF-2. These GFs and ILs were selected for their specific epitheliotrophic role in the ocular surface epithelial cell. Samples were thawed before each assay, taking the necessary amount for each test. Samples were evaluated using commercially available multi-plex bead-based sandwich immunoassay kits (Bio-Rad Laboratories, CA, USA), by means of the Bio-Plex Protein Array System (Bio-Rad Laboratories, CA, USA) as previously described [21]. This system allows simultaneous quantitative analysis of multiple different factors in a single microtiter well. Values with a coefficient of variation above the 10% were discarded before the final data analysis. Data were analyzed by the Bio-Plex Manager software version 6.0 (Bio-Rad Laboratories, CA, USA). Standard levels between 70 and 130% of the expected values were considered to be accurate and were used.

2.6. Statistical analysis

Statistical analysis was performed with computer software (SPSS, Version 14.0, SPSS, Inc., Chicago, IL, USA) and MedCalc 5.0, (MedCalc Inc, Ostend, Belgium). Descriptive statistics for tests and variables analyzed in subjects were reported as median, maximum and minimum values, 5–95 percentiles distribution. Comparison among GFs levels in the two blood sources was performed with Mann-Whitney test for unpaired data with a p value of less than 0.05 was considered to be significant. Spearman's (ρ) correlation coefficient was calculated, correlations were considered statistically significant at $p < 0.05$ and a correction of p-values for multiple testing was introduced. Strength of correlation ranged from -1 to +1 and was estimated in absolute values as 0-0.19 "very weak"; 0.20-0.39 "weak"; 0.40-0.59 "moderate"; 0.60-0.79 "strong"; 0.80–1.0 "very strong" [22].

3. Results

3.1. Characteristics of blood samples

A total of 107 CB samples was collected from spontaneous term births free of complications (≥ 37th week of pregnancy, n = 87) and Caesarean births (n = 20) The characteristics of mothers and babies are reported in Table 1a, divided by mother and baby donors.

A total of 105 PB samples was collected from adult healthy subjects, the donor characteristics are reported in Table 1b. All the CB-S and PB-S samples were analyzed as hereby described.

3.2. Growth factors and interleukin data

The growth factors and interleukins levels in CB-S and PB-S are reported in Table 2. The levels of EGF, TGF-α, TGF-β2, FGF, PDGF, VEGF and NGF were significantly higher in CB-S samples compared to PB-S samples. Conversely, the levels of IGF-1, IGF-2 and TGF-β1 were significantly higher in PB-S samples (Fig. 1). No significant difference was found in the level of TGF-β3. The more represented GFs were TGF-β1, PDGF and TGF-β2 in both CB-S and PB-S. The composition of the other GFs varied between the two blood sources (Fig. 2). The levels of IL-1β, IL-4, IL-6, IL-10, and IL-13 were significantly higher in CB-S samples as compared to PB-S samples.

3.3. Correlation analysis

A correlation analysis was performed to compare the level of each GF. Correlation coefficients for both blood sources are reported in