TITLE

Comparative Effects of Autologous Serum, Umbilical Cord Blood-Derived Drops, and Platelet Lysate on Ocular Surface Parameters

Date: October 18 2023

ABSTRACT

Dry eye disease is a multifactorial disorder of the ocular surface that can significantly affect a patient's quality of life. Its treatment depends on the severity of symptoms and usually includes the use of artificial tears or lubricating ointments, while anti-inflammatory eye drops, punctal collagen plugs, or autologous serum drops are used less frequently. Growth factors derived from platelets—either from peripheral blood or umbilical cord blood—such as TGF-β1, PDGF-αα/ββ, FGF-1, and VEGF-α/β, represent new therapeutic approaches that have been successfully applied in regenerative medicine and wound healing.

The aim of this dissertation is to evaluate the application of eye drops derived either from autologous peripheral blood or from umbilical cord blood, and to compare their safety and effectiveness with autologous serum eye drops (three patient groups, age >18 years, diagnosed with ocular surface disease, n = 34).

1.INTRODUCTION

Dry eye disease is a common and multifactorial disorder of the ocular surface that can significantly affect a patient's quality of life due to ocular discomfort and visual disturbances.[1] Its global prevalence ranges from 5% to 50% and increases with age.[2] Patients with dry eye disease can be classified into four categories depending on the severity of their clinical presentation, which may be mild, moderate, severe, or very severe. Dry eye can cause itching, burning, foreign-body sensation, intermittent blurred vision, or even diplopia. The eyes are often red and irritated due to reduced tear production or poor tear quality.[1] Major aggravating factors include eyelid margin inflammation (blepharitis), aging, diet, environmental factors such as air conditioning and low humidity, female sex in association with hormonal changes (pregnancy, menopause), medications (antihistamines, contraceptives), and systemic diseases (rheumatoid arthritis).[3]

Treatment of dry eye depends on symptom severity and includes artificial tears or lubricating ointments, punctal collagen plugs, anti-inflammatory eye drops, or autologous serum drops. However, surgical intervention may be required to correct eyelid malposition when the eyelid does not close properly ("lagophthalmos").[3] The use of autologous serum was first described in the international literature as a therapeutic option for ocular burns in 1975.[4] The preparation of eye drops for treating dry eye in patients with Sjögren's syndrome was first reported in 1984[5] and remains one of the main therapeutic tools for ocular surface disease.[6] However, autologous serum does not contain platelets, which are rich in important growth factors. Harnessing these growth factors represents one of the most advanced therapeutic approaches for disorders of skin wound healing (e.g., epidermolysis bullosa, diabetic foot, deep dermal burns). [7–11]

For this purpose, modern techniques have been developed to more effectively isolate and deliver platelet-derived growth factors.[12] Blood derivatives rich in growth factors are obtained after centrifugation of whole blood and are classified as:

(a) platelet-rich plasma (PRP), (b) platelet-poor plasma (PPP), (c) platelet lysate (PL), and (d) platelet gel (PG).[13]

In PRP, platelet concentration is 3–5 times higher than normal blood values (peripheral or umbilical cord).[14–15] The release of platelet-derived growth factors occurs either after platelet activation (via exogenous activators such as calcium chloride), which leads to secretion of α-granule contents, or through complete platelet lysis (freeze–thaw cycles).[16] When the latter method is used, the final product is termed PL.

The main biomolecules contained in PL with beneficial effects on tissue regeneration include growth factors such as TGF-β1, PDGF-αα/ββ, FGF-1, VEGF-α/β, HGF, IGF, EGF; anti-inflammatory cytokines such as IL-1ra, IL-10, IL-13; and immunomodulatory elements such as galectins, IDO, NO, HGF, among others.[17] These biomolecules can appropriately activate stem cells, fibroblasts, and progenitor cells to migrate to the site of injury and promote tissue regeneration, as well as regulate inflammatory processes, further supporting tissue repair.[18]

The use of these products has been applied with remarkable success in extensive lower-limb ulcers in diabetic patients (“diabetic foot”), in patients with second- and third-degree skin burns, and in dystrophic ulcers caused by epidermolysis bullosa.[19–20] The healing outcomes of these wounds are attributed to (1) the rapid release of platelet-derived growth factors (such as TGF-β1, FGF, VEGF, HGF, PDGF, IGF, etc.), (2) the paracrine action of these factors on various cell populations (epithelial, endothelial, mesenchymal cells and fibroblasts), and (3) the activation of signaling pathways related to cell proliferation, production of structural proteins, and wound healing.[7–8]

In the international literature, the successful use of PL has been described in patients with ocular surface disease, corneal ulcers, and graft-versus-host disease (GVHD).[21][23]

2.AIM

The aim of the present dissertation is to evaluate the application of eye drops derived either from autologous peripheral blood (peripheral blood eye drops – PBED) or from umbilical cord blood (cord blood eye drops – CBED), and to compare their safety and effectiveness with autologous serum eye drops in patients with ocular surface disease.

It has been reported in the literature that umbilical cord blood may contain a greater number of immunomodulatory components compared to peripheral blood, due to circulating biomolecules such as HLA-G. [17] These biomolecules are responsible for maternal tolerance toward the semi-allogeneic fetus. Additionally, patients with cardiac or metabolic diseases, or those receiving treatments that affect platelet levels, produce PL of reduced quality with respect to its biomolecular content. For such patients, the use of blood-derived products of allogeneic origin may represent the best possible option.

It is particularly noteworthy that PL production is achieved through simple centrifugation and freeze–thaw cycles, and is therefore classified as a minimally manipulated product according to paragraph 21 CFR of the FDA.[22] Consequently, its production and administration do not fall under the category of pharmaceutical products and thus do not require special authorization from the National Organization for Medicines (EOF) for broad clinical study.

3.METHODS

In this prospective randomized study, eye drops will be prepared and administered using either autologous peripheral blood from the patients or umbilical cord blood.

3.1 Preparation of platelet lysate eye drops from autologous peripheral blood

The processing and preparation of eye drops derived from autologous peripheral blood will take place in an appropriate facility at GNA Gennimatas Hospital, ensuring sterile production conditions and minimizing the risk of contamination of the final product by laboratory personnel.

For the production of these eye drops, an initial blood volume of 50 ml is required from each patient. The blood is collected in standard blood collection tubes containing anticoagulant, followed by centrifugation at 900 g for 10 minutes to isolate PRP. The PRP is diluted with sterile saline to a final concentration of 30% (v/v), collected in sterile 1.5 ml containers, frozen at –80°C for at least 60 minutes (thermal shock), and rapidly thawed at 4°C to produce PL. The final PL product is then refrozen at –20°C and stored in the patients' freezer for approximately 45 days. On the day of administration, the PL is rapidly thawed at 4°C and can be immediately used as an eye drop.[23]

After production, the eye drops are placed in sterile 5 ml and 10 ml dropper bottles to ensure easy application to the patients' eyes.

It is estimated that an initial 50 ml blood draw yields approximately 7.5 ml of PL, which can be used directly as eye drops for ocular diseases.

For eye drops derived from autologous peripheral blood, additional testing is required for blood-borne infectious diseases, specifically HIV I/II, HCV, HAV, HBV, HCV, HTLV I/II, WNV,

T. pallidum, T. cruzi, and CMV (IgM and IgG). If any of these are present, the patient must report them in the study consent form.

Further evaluation of the production process includes sterility testing for anaerobic and aerobic microorganisms as well as fungi using the BacT/Alert system. All tests are performed according to the guidelines of the European Pharmacopoeia (European Pharmacopeia, PBI S.p.A., Milan, Italy) to ensure sterility of the final product.[24]

3.2 Preparation of platelet lysate eye drops from umbilical cord blood

The processing and preparation of eye drops derived from umbilical cord blood will take place in Class B Good Manufacturing Practice (GMP) rooms at the ELTOPA laboratory, ensuring sterile production conditions and minimizing the risk of contamination of the final product.

After production, the eye drops are placed in sterile 5 ml and 10 ml dropper bottles to ensure easy application to the patients' eyes.

The production of eye drops from umbilical cord blood follows the PL preparation protocol published by the Hellenic Cord Blood Bank (ELTOPA)[25–26] and is performed using cord blood units from full-term pregnancies (38–40 weeks, vaginal delivery or cesarean section) that are not eligible for processing and transplantation according to FACT-NetCord accreditation criteria. All units received by ELTOPA are accompanied by signed maternal consent for cord blood donation, obtained prior to delivery and in accordance with the National Bioethics Committee.

Cord blood units undergo double centrifugation followed by PRP isolation. The PRP is then stored at –80°C for at least 24 hours, rapidly thawed at 37°C to fully release platelet-derived growth factors, and used to produce PL.

The PL is then filtered using a pyrogen-free 0.22 μm filter to remove cellular debris and stored in 5–10 ml quantities. Typically, up to 10 ml of PL can be produced from one cord blood unit.

Eligibility criteria for cord blood–derived eye drops include: PLTs > 15×10^9/L, WBCs < 4×10^9/L, RBCs < 0.1×10^{12}/L, and absence of hemolysis (<0.8%). CBED can be stored at –20°C for 30 days without changes in platelet-derived growth factor content.

Before distribution, CBED undergo testing for blood-borne infectious diseases: HIV I/II, HCV, HAV, HBV, HCV, HTLV I/II, WNV, T. pallidum, T. cruzi, and CMV (IgM and IgG).

Sterility testing for anaerobic and aerobic microorganisms and fungi is also performed using the BacT/Alert system. All tests follow the European Pharmacopoeia guidelines to ensure sterility of the final product.

3.3 Preparation of eye drops from autologous serum

The processing and preparation of autologous serum eye drops will take place in an appropriate facility at GNA Gennimatas Hospital, ensuring sterile production conditions and minimizing contamination risk.

For the production of autologous serum eye drops, 50 ml of blood is required from each patient, along with preservative-free saline. The blood is collected in standard tubes and centrifuged at 4000 g at +8°C for 15 minutes. Then, 3.5 ml of saline eye drops are removed and replaced with 3.5 ml of autologous serum.[6]

Autologous serum eye drops require additional testing for blood-borne infectious diseases (HIV I/II, HCV, HAV, HBV, HCV, HTLV I/II, WNV, T. pallidum, T. cruzi, CMV IgM/IgG). If present, the patient must report them in the consent form.

The final product can be stored for one week at +4°C (refrigeration) and for four weeks at –20°C (freezing).

3.4 Statistical Power Analysis (G-Power Analysis) and Data Evaluation

Data analysis for all study parameters will be performed using GraphPad Prism v6.01 (GraphPad Software, San Diego, CA, USA). Parameter analysis will include one-way ANOVA, non-parametric Kruskal Wallis, Mann-Whitney and Wilcoxon tests.. Statistical significance is set at $p < 0.05$.

3.5 Study Groups

This protocol proposes evaluating the healing capacity of eye drops derived from PL of autologous peripheral blood and umbilical cord blood compared with autologous serum. Specifically, three patient groups will be assessed:

Group 1: Patients (n = 34) with diagnosed ocular surface disease receiving PL eye drops from autologous peripheral blood. Recommended dosage: 1–3 drops, four times daily for 4 weeks.

Group 2: Patients (n = 34) with diagnosed ocular surface disease receiving PL eye drops from umbilical cord blood. Recommended dosage: 1–3 drops, four times daily for 4 weeks.

Group 3: Patients (n = 34) with diagnosed ocular surface disease receiving autologous serum eye drops (already used in our clinic). Recommended dosage: 1–3 drops, four times daily for 4 weeks.

3.6 Evaluation of Eye Drop Application

The effectiveness of the therapeutic interventions will be assessed using specialized ophthalmic techniques. Epithelialization time is defined as the interval from treatment initiation to complete epithelial healing.

For qualitative assessment of tear film stability, the tear breakup time (TBUT) will be measured—the interval between the last blink and the appearance of the first dry spot after instillation of 2% fluorescein. A TBUT < 5 seconds is indicative of ocular surface disease.

For quantitative assessment of tear production, the Schirmer test will be used, measuring the wetting length of a 5 mm × 35 mm filter paper strip (No. 41 Whatman). The strip is placed at the junction of the middle and outer third of the lower eyelid, and the patient closes their eyes for 5 minutes. The test may be performed without anesthesia (Schirmer I) or with anesthesia (Schirmer II). Wetting < 10 mm (Schirmer I) or < 6 mm (Schirmer II) is considered abnormal.

Corneal epithelial damage will be assessed using fluorescein staining. Tear meniscus height—and thus tear volume—will also be measured.

To evaluate subjective symptoms, each patient will complete the Ocular Surface Disease Index (OSDI) questionnaire, consisting of 12 questions assessing symptoms and their impact on daily life. It includes three subcategories 1) ocular symptoms, 2) vision-related daily functioning, 3) environmental triggers. Responses range from 0 (“none of the time”) to 4 (“all of the time”). Total scores range from 0–100: 0–12 = normal, 13–22 = mild disease, 23–32 = moderate disease, 33 = severe ocular surface disease.[27]

Inclusion Criteria

- Age > 18 years
- Ocular surface disease due to chronic blepharitis
- Ocular surface disease due to Sjögren’s syndrome
- Toxic keratopathy from antiglaucoma drops
- Persistent epithelial defect after ocular surgery (e.g., keratoplasty)
- Chemical burn
- Post-herpetic keratitis

Exclusion Criteria

- Age < 18 years
- Active infectious disease

4.DISCUSSION

In the present doctoral dissertation, a comparative study will be conducted for the first time in the international literature using eye drops derived from autologous peripheral blood or umbilical cord blood in patients with ocular surface disease.

The expected outcomes of this study include healing of the injured eye and an increase in the number of epithelial cells within a shorter period of time in patients belonging to Groups 1, 2 and 3.

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