A Life that is born makes Life Grow
Cord Blood: current experiences and future programmes

Biological eye drops

Marina Buzzi
Emilia-Romagna Cord Blood Bank
SIMT A.M. BO.
Policlinico S. Orsola- Malpighi - University of Bologna
INTRODUCTION

• Failure of the ocular surface epithelium occurs in many clinical conditions with differing pathogeneses; the core of medical management is to stabilize or promote healing of healthy corneal epithelium.

• Dry eye (DE) syndrome is a very common disorder that may also represent a sight threatening disease and a potential cause of blindness as irreparable ulceration, perforation or scarring can also occur in untreated or uncontrolled severe cases.

• First line treatment in DE syndrome, including the application of artificial tears, topical anti-inflammatory agents, therapeutic contact lenses and punctual occlusion, lacks the biologically active components present in normal tears that are critical for a healthy epithelium
Biological eye drops

• Blood derived Biological eye drops, including autologous serum (AS), platelet rich plasma (PRP), and homologous serum, like cord blood serum (CBS), have been introduced in the treatment of many ocular diseases, because they contain a high concentration of biologically active components (mucin, neuropeptides, fibronectin, vitamin A, alpha 2 macroglobulin) and growth factors (EGF, TGF beta1), that are usually found in tear film and are essential for regulating the proliferation, differentiation and maturation of ocular surface epithelium.

• These natural components may support the healing of injured ocular surface epithelium, in case of diminished tear growth factor content, as may occur in DE disease.
Biological eye drops

• More recently some Authors have suggested what could be the next generation of biological eye drop therapy for severe ocular surface disease: eye drops derived from human mesenchymal stem cell (hMSC)-conditioned medium, that may have antiapoptotic and/or proepithelial wound healing effect via a paracrine mechanism. (Oh JY et Al. Invest Ophtalmol Vis Sci 2014; Roddy GW et Al. Stem Cells 2011).

• The challenge for researchers in this field will be to identify and test the optimum hMSC culture conditions and protocols to produce safe, tolerable and effective hMSC conditioned medium eye drops without the proangiogenic factors also known to be produced by these cells.
Process of corneal damage repair

Klenkler B et al., Exp Eye Res. 2004
<table>
<thead>
<tr>
<th>NS-NSAID</th>
<th>Cord Blood Serum</th>
<th>Autologous Serum</th>
<th>Tear Substitutes</th>
<th>NSAID, Steroids</th>
<th>Cyclosporin A</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH/Osmolarity</td>
<td>natural</td>
<td>natural</td>
<td>chemically buffered controlled</td>
<td>chemically buffered controlled</td>
<td>chemically buffered controlled</td>
</tr>
<tr>
<td>Preservatives</td>
<td>free</td>
<td>free</td>
<td>present</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Patients compliance</td>
<td>optimal</td>
<td>optimal</td>
<td>good depending upon type</td>
<td>products burn</td>
<td>products burn</td>
</tr>
<tr>
<td>Chemical components</td>
<td>absent</td>
<td>absent</td>
<td>present</td>
<td>present</td>
<td>present</td>
</tr>
<tr>
<td>Ocular nutrients</td>
<td>present</td>
<td>present</td>
<td>present only in the last generations of products</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Growth factors</td>
<td>present</td>
<td>present</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Anti-inflammatory properties</td>
<td>present, natural substances (direct effects)</td>
<td>absent (presence of pro-inflammatory cytokines in PBST)</td>
<td>absent (only diluting, indirect effects)</td>
<td>present, chemical compounds</td>
<td>present, chemical compounds</td>
</tr>
</tbody>
</table>
Autologous eye drops

• The use of AS in the ophthalmic setting dates back at least two decades, but despite its proven efficacy there is no standard procedure for preparation, quality control, storage and administration.
Autologous eye drops

• In more recent years, the platelet lysate obtained from autologous platelet-rich plasma has attracted increasing interest because it is also a source of a variety of growth factors with an important role in the wound-healing process in many tissues.


The role of "eye platelet rich plasma" (E-PRP) for wound healing in ophthalmology.

Alio JL, Amalich-Montiel F, Rodriguez AE.


Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis.

Kim KM, Shin YT, Kim HK.
• The authors identified four eligible randomized controlled trials (RCTs) among 30 studies reviewed, in which AS was compared with artificial tear treatment or saline in individuals with DE of various etiologies.

• The meta analysis showed inconsistent results for AS efficacy.

• Well-planned, large scale, high quality randomized controlled trials are needed, stratified by age and severity of DE, comparing AS to artificial tears or other treatments, as well as evaluating various concentrations of AS.
Homologous eye drops

- Homologous blood sources (from adult blood donors and from cord blood donors) have recently been proposed, with the advantage that they can be routinely obtained as quality/microbiologically-controlled products from blood banks, where a supply may be kept and stored in advance to be readily dispensed.
Homologous eye drops

• Use of homologous products may also enable us to treat patients with serious immunologically jeopardised diseases associated with DE.

• These would be damaged by autologous products which contain non homogeneous levels of GFs and high levels of pro-inflammatory cytokines.
CBS eye drops

• Several authors have demonstrated the safety and efficacy of CBS eye drops in the treatment of severe DE with or without Sjogren’s syndrome (SS-I), ocular Graft versus host disease (GVHD), persistent corneal epithelial defects, recurrent corneal erosions, chemical burns and neurotrophic keratitis.

• CBS eye drops seem to be more effective than AS in decreasing symptoms and keratoepitheliopathy in patients with severe DE syndrome, and increasing goblet cell density in SS patients.
Comparison of Autologous Serum and Umbilical Cord Serum Eye Drops for Dry Eye Syndrome

KYUNG-CHUL YOON, HWAN HEO, SEONG-KYU IM, IN-CHEON YOU, YOON-HA KIM, AND YEOUNG-GEOL PARK

AMERICAN JOURNAL OF OPHTHALMOLOGY

JULY 2007

T0 AS (day 0)

T0 CBS (day 0)

T1 AS (day 60)

T1 CBS (day 60)
CBS eye drops

• Some other studies evaluating the effects of CBS eye drops in treating corneal epithelial defects, have given encouraging results.

• The studies confirm that CBS contains essential substances for the proliferation, differentiation and maturation of the normal ocular surface epithelium, like Epidermal growth factors (EGF), vitamin A and Transforming growth factor -β.1 (TGF-β.1),

• Epidermal growth factor in particular plays a central role in corneal wound healing
Application of Umbilical Cord Serum Eyedrops for the Treatment of Dry Eye Syndrome

Kyoung-Chul Yoon, MD, PhD,* Seong-Kyu Im, MD, * Yeong-Geol Park, MD, PhD, * Young-Do Jung, MD, PhD, † Seong-Yeul Yang, MD, PhD, † and Jin Choi, MD, PhD ‡

Cornea • Volume 25, Number 3, April 2006

Use of umbilical cord serum were observed. The mean concentrations of EGF, TGF-β, and vitamin A in umbilical cord serum and peripheral blood serum are shown in Table 2. EGF and TGF-β concentrations of umbilical cord serum were significantly higher than those of peripheral blood serum. Vitamin A concentration of umbilical cord serum was significantly lower than that of peripheral blood serum.


Application of umbilical cord serum eyedrops for recurrent corneal erosions.

Yoon KC†, Choi W, You IC, Choi J.

CONCLUSIONS: In the treatment of recurrent corneal erosions, umbilical cord serum eyedrops may be effective in reducing the number of recurrences.
Use of Umbilical Cord Serum in Ophthalmology

Kyung Chul Yoon

Owing to the high concentrations of essential tear components, growth factors, and neurotrophic factors in UCS, UCS eye drops can be safely and effectively applied in intractable ocular conditions such as severe dry eye disease with or without Sjögren's syndrome, ocular GVHD, persistent epithelial defects, neurotrophic keratopathy, recurrent corneal erosions, ocular chemical burn, and surface problems after corneal refractive surgery.

Umbilical cord blood serum therapy for the management of persistent corneal epithelial defects

Elif Erdem, Meltem Yagmur, Inan Harbiyeli, Hande Taylan-Sekeroglu, Reha Ersoz
Efficacy of Standardized and Quality-Controlled Cord Blood Serum Eye Drop Therapy in the Healing of Severe Corneal Epithelial Damage in Dry Eye

Piera Versura, BSD,* Vincenzo Profazio, MD,* Marina Buzzi, BSD, † Alessandra Stancari, PharmD, † Mario Arpinati, MD, § Nazzarena Malavolta, MD, ¶ and Emilio C. Campos, MD,*

Purpose: We standardized quality-controlled cord blood serum (CBS)–based eye drops and evaluated the efficacy of 1-month CBS treatment in the healing of diseased corneal epithelium in severe dry eye.

Conclusions: Heterologous CBS-based eye drops represent a promising therapeutic approach in the healing of severely injured corneal epithelium and in subjective symptom relief. These drops can be obtained as readily available and quality-controlled blood derivative from cord blood banks on a routine basis.
First European trial on standardized preparation and clinical evaluation of CBS eye drops

Oftalmology Unit, Bologna University

Emilia-Romagna cord blood bank (ERCB) Transfusion Service

Pharmacy Service

EudraCT: 2008-005757-38
Clin Trial Gov Id NCT01234623

Seventeen GVHD and thirteen SS-I patients were included in the study. They had experienced AS therapy in their previous clinical history, exhibiting severe corneal involvement, at the time of enrolment, graded according to the Oxford grading level (16). Informed consent was obtained from each enrolled subject.
Inclusion and exclusion criteria

**Inclusion**

- GVHD and SS type I with severe corneal epithelial defects (Oxford grade 5)
- resistant to or dependent on topical steroids
- resistant or unable to receive autologous serum

**Exclusion**

- previous (one year) ocular surgery
- contact lenses
- punctal plugs or cauterization

50% of the patients had been treated with topical Cyclosporine A

The entire cohort had to stop all concomitant treatments, except for tear substitutes, 4 days before starting CBS therapy (washout period).
Standardization of eye drop preparation

- A mean volume of 80 ml of CB was collected from the umbilical vein and clotted for two hours at room temperature. After centrifugation at 3,800 g for 10 minutes, the serum has isolated under a laminar flow hood and frozen at -80°C. In the other case blood samples were collected from ex utero placental vessels with a sterile syringe and transferred into Vacutest tubes without any anticoagulant.

- Sterility tests demonstrated that all batches remained sterile after handling and storage. The CB serum levels of EGF, TGF-β1 were maintained throughout the process.

<table>
<thead>
<tr>
<th>Sample</th>
<th>EGF Content (pg/ml)</th>
<th>TGF-β1 Content (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2300</td>
<td>80</td>
</tr>
<tr>
<td>B</td>
<td>2100</td>
<td>85</td>
</tr>
<tr>
<td>C</td>
<td>1900</td>
<td>70</td>
</tr>
<tr>
<td>D</td>
<td>1700</td>
<td>65</td>
</tr>
<tr>
<td>E</td>
<td>1500</td>
<td>60</td>
</tr>
<tr>
<td>F</td>
<td>1300</td>
<td>55</td>
</tr>
<tr>
<td>G</td>
<td>1100</td>
<td>50</td>
</tr>
</tbody>
</table>

Specifically as follows: 1: freshly collected CBS; 2: after the quarantine period; 3: after dilution; 4: after filtration; 5: after 1 month of freezing; 6: after 2 months of freezing. A considerable biological variability was shown in both EGF (700–2300 pg/ml) and TGF-β1 (800–1900 ng/ml) across the samples analyzed, but no significant variation was observed throughout the whole process, suggesting that the procedure does not affect or reduce the content of both growth factors.
EGF and TGF β1 dosage

The concentration of EGF and TGF β1 in CB units was determined using a Quantikine Human EGF Immunoassay Kit according to manufacturer’s instructions.

Graphics show the difference in concentration of EGF and TGF- β1 among normal subjects, SS-1 patients, GVHD patients and Cord Blood Serum. Note that the concentration of EGF and TGF- β1 in SS-1 and GVHD patients is less than normal subjects while in the case of Cord Blood Serum it is higher.
Targeting growth factor supply in keratopathy treatment: comparison between maternal adult peripheral and cord blood sources for preparation of topical eye drops.

Versura P¹, Buzzi M², Giannaccare G¹, Terzi A², Fresina M¹, Velati C², Campos EC¹

Results EGF, TGF-β and VEGF levels were significantly higher in CB-S than in PB-S (median respectively 1254.4 vs 646.0 pg/ml, 51.3 vs 38.4 µg x ml and 686.8 vs 30 pg x ml, always p<0.0001) whereas IGF content was significantly higher in PB-S than in CB-S (respectively 159.9 vs 53.5 pg x ml, p<0.0001). In CB-S, CD34+ cell concentration appeared to be related to EGF, IGF and TGF-β levels whereas WBC appeared in relationship with EGF and TGF-β levels. VEGF levels showed no relation with the hematological parameters considered. PLT Discussion. Data showed a differential GFs content in the two blood sources, with a higher contribution from CB. Each GF selectively regulates cellular processes involved in corneal healing, so the use of PB or CB should be targeted for specific GF supply on the basis of type and severity of keratopathy.

Conclusive comment this study showed that EGF and TGF-β supply may be significantly higher in CB-S as primary source. CB-S is also the selected choice for products to treat corneal nerve damage as VEGF supply from PB-S may be negligible. Conversely, PB-S can be indicated as primary source for products to accelerate keratinocyte metabolism and heal stromal defects, due to its higher IGF content.
Standardization of eye drop preparation

• We decided to standardise EGF content in CBS eye drops.
• A certain variability in EGF levels in CBS may be present and a preliminary dosage of EGF content in CB samples should be performed to control the amount of EGF in the final eye drops.
• CBS with EGF > 1.0 ng/ml was selected as the threshold
• This allows us to avoid variability of composition and improve the homogeneity of clinical results analysis.
Standardization of eye drop preparation

• We demonstrated the healing efficacy of CBS eye drops prepared according to a standardised protocol focusing on obtaining a daily supply of 0.10-0.20 ng/mL EGF, similar to the physiological human tear content.

• The preselected sera were thawed and pooled to obtain the amount of serum needed to treat all patients, diluted to 20% with phosphate buffered saline by an aseptic technique, and filtered (Millex HV 0.4 µm). The preparation was then aliquoted in monodose vials using a COL-20 medical device (Biomed Italy), before being packed frozen and stored.
• Patients were instructed to administer one drop per eye for 8 times a day, after having thawed 1 vial the evening before the day of use.
Patients were evaluated for **corneal epithelial damage** and for **discomfort symptoms** at baseline (Visit 0-V0), after 15 (V1), after 30 (V2) days of treatment.
**Results**

**OSDI**

- Baseline V0: 39.3 ± 16.9
- V1: 24.4 ± 10.3
- Endpoint V2: 22.3 ± 10.3

- p < 0.0001

**Schirmer test**

- Baseline: 2.64 ± 2.31
- Endpoint: 3.38 ± 2.05

- p = 0.0001

**BUT**

- Baseline: 5.1 ± 3.2
- Endpoint: 5.7 ± 3

- p < 0.0001

**Corneal esthesiometry**

- Baseline: 48.22 ± 2.85
- Endpoint: 49.66 ± 2.42

- p < 0.0001
Results

Tear osmolarity

Baseline: 322 ± 9 mOsm/L
Endpoint: 312.5 ± 7.3 mOsm/L

p < 0.0001

Scraping cytology

Baseline: 6.6 ± 2.1
Endpoint: 3.8 ± 1.2

p < 0.0001

Imprint cytology

Baseline: 1.89 ± 0.45
Endpoint: 1.64 ± 0.51

P = 0.0002
Results

![Graph showing the damaged corneal epithelium areas at baseline (V0), V1, and endpoint (V2)].

Baseline V0: 40.9 ± 30
V1: 23.5 ± 17.3
Endpoint V2: 16.1 ± 13.7

Significance levels:
- P < 0.0001
- P = 0.06

F, 30aa, R.B. SS-I
Satisfaction questions estimated by a VAS score

My eyes feel dry in the morning
- V1: 6.0 ± 1.5
- Endpoint V2: 4.5 ± 1.2
- P = 0.0009

My eyes feel dry at the end of the day
- V1: 6.5 ± 1.3
- Endpoint V2: 5.0 ± 1.0
- P = 0.0001

My eyes feel refreshed when I use UCS drops
- V1: 7.0 ± 2.0
- Endpoint V2: 8.5 ± 1.5
- P < 0.0001

My eyes feel refreshed longer than expected, when I use UCS drops
- V1: 8.0 ± 1.2
- Endpoint V2: 8.5 ± 1.0
- P = 0.0008
Satisfaction questions estimated by a VAS score

P=0.0002

I frequently forgot my symptoms during the use of UCS drops

whole satisfaction report
Limited extent of initial corneal damage is the SOLE factor predicting response

Duration of disease, Systemic GVHD, IS treatment and TBI DO NOT correlate with response to CBS therapy.
Cord blood treatments
patient number/diseases

Updated May 2015

Pain symptom reduction by 50% in the first week

Number of treatments/patients
Complete healing 35 out of 69 patients
Reduction over 50% defect 30 out of 69 patients
Reduction less 50% defect 4 out of 69 patients
CONCLUSIONS

*Standardized pooled CB Serum Eye Drops:*

- Are safe
- Reduce corneal damage
- Reduce inflammation
- Improve tear production
- Reduce discomfort symptoms
- Have limited duration
CBS eye drops has significant advantages over amniotic membrane:

- can be used in patients with poor general health and those unfit for surgery
- can be used in children in whom can also be avoided unnecessary general anesthesia
- can be administered for prolonged periods whereas, amniotic membrane is retained on the ocular surface for only a limited time period and this may limit the total exposure and availability of the growth factors to the ocular surface
Where do we could go in the future .......?
MAINTENANCE THERAPY WITH REPEATED CYCLES OF CBS EYE DROP TREATMENT...

EVALUATE THE EFFICACY OF TWO OMOLOGOUS BLOOD SOURCE (ADULT PERIPHERAL BLOOD AND CORD BLOOD) AS GROWTH FACTOR SUPPLY IN THE HEALING OF SEVERE CORNEAL EPITHELIAL DEFECTS WITH A PROSPECTIVE, RANDOMIZED DOUBLE BLIND STUDY
Thanks

ERCB - SIMT AM BO – Dr. Claudio Velati
Adriana Terzi
Cristiana Vaselli
Elisa Bergantin

U.O. Oftalmologia – Prof. Emilio Campos
Piera Versura
Vincenzo Profazio
Giuseppe Giannaccare