

ORIGINAL ARTICLE

Therapeutic effect of umbilical cord serum eyedrops for the treatment of dry eye associated with graft-versus-host disease

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This study was performed to investigate the therapeutic effect of umbilical cord serum eyedrops on dry eye associated with graft-versus-host disease (GVHD). Twenty-four eyes of 12 patients with severe dry eye syndrome associated with GVHD were treated with 20% umbilical cord serum eyedrops. Symptom scoring, corneal sensitivity test, tear film break up time (BUT), Schirmer test, tear clearance rate (TCR), and corneal fluorescein staining were performed before and 2 and 6 months after treatment. Six months after treatment, significant improvement was observed in symptom score (from 3.83 ± 0.38 to 0.83 ± 0.57 , $P < 0.01$), corneal sensitivity (from 52.08 ± 6.06 mm to 57.50 ± 3.00 mm, $P < 0.01$), tear film BUT (from 2.50 ± 0.91 s to 5.71 ± 1.04 s, $P < 0.01$), and keratoepitheliopathy score (from 7.42 ± 2.02 to 1.29 ± 0.46 , $P < 0.01$). There was no significant change in Schirmer test and TCR results. No significant complications associated with the use of the eyedrops were observed. Umbilical cord serum eyedrops are safe and may be an effective way to treat severe dry eye associated with GVHD.

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Introduction

Acute or chronic graft-versus-host disease (GVHD) can lead to various ocular diseases. Among these, dry eye develops most frequently and can result in serious ocular complications such as punctate keratitis, persistent epithelial defects, keratinization of the cornea, corneal ulceration, and perforation.^{1–3} Dry eye associated with

GVHD is known to be difficult to treat with conventional treatment including the application of artificial tears, therapeutic contact lenses, protective goggles or anterior chamber glasses, punctal occlusion, topical retinoic acid, topical or systemic corticosteroids, and immunosuppressive agents such as cyclosporin A and FK 506.^{1–6}

Autologous serum contains substances like epidermal growth factor (EGF), vitamin A, transforming growth factor- β (TGF- β), fibronectin, and other cytokines that are essential for the maintenance of the corneal and conjunctival epithelia and has been used effectively to treat severe dry eye in patients with chronic GVHD.^{7–9} Recently, we found that umbilical cord serum also contains many growth factors and essential tear components, and umbilical cord serum eyedrops are safe and effective for treating dry eye syndrome and persistent epithelial defect.^{10–11}

In the present study, we prospectively investigated the therapeutic effect of umbilical cord serum eyedrops on severe dry eye associated with GVHD.

Patients and methods

Patients with dry eye syndrome associated with GVHD who were refractory to conventional treatments and had low tear film break up time (BUT, < 5 s), low Schirmer test (5 mm), and positive fluorescein or rose bengal vital staining (≥ 3) were included.¹² Individuals who had an active ocular infection or inflammation not associated with dry eye, drug toxicity and abnormalities in the eyelid or eyelashes were excluded from this study.

Twelve patients (24 eyes) were recruited for this study. Informed consent was obtained from each subject enrolled in this study. Institutional review board/ethic committee approval was obtained from the Institutional Review Board, and the study protocol followed the guidelines of the Declaration of Helsinki.

The umbilical cord blood was obtained from mothers with vaginal or caesarean section delivery after obtaining informed consent. Laboratory examination for hepatitis B and C virus and human immunodeficiency virus (HIV) were performed twice at 8 and 38 gestational weeks. A volume of 200–250 ml of the umbilical cord blood was collected from the umbilical vein after fetal delivery. The blood was clotted for 2 h at room temperature. After

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centrifugation at 3000 r.p.m. for 15 min, the serum was isolated carefully and then diluted with balanced salt solution (BSS, Alcon, Forth Worth, TX, USA) at a concentration of 20%. Diluted serum was aliquoted into sterile 5 ml bottles with ultraviolet light protection. Patients were instructed to keep an opened bottle in a refrigerator (4°C) for 1 month and to store unopened bottles in a freezer (-20°C) for 3 months. Patients were also instructed to instill umbilical cord serum eyedrops 6–10 times a day in addition to the prior artificial tears.

Symptom scoring, corneal sensitivity test, tear film BUT, Schirmer test, tear clearance rate (TCR) and corneal fluorescein staining were performed by the same researcher before and at 2 and 6 months after treatment.

Subjective symptoms were graded on a numerical score of 0–4, with 0 representing no symptom and four representing very severe symptoms that caused discomfort and interfered with normal activities. Corneal sensitivity was measured using a Cochet-Bonnet esthesiometer (Luneau, Chartres, France). The tip of the fully extended nylon filament was applied perpendicular to the surface of the central cornea and advanced steadily. When the subject felt its presence, the length of the filament was recorded in millimeters.

Tear film BUT and Schirmer test with topical anesthesia were measured as described.¹³ TCR was performed 5 min after instilling a 10 μ l drop of 0.5% fluorescein and 0.4% oxybuprocaine hydrochloride into the conjunctival sac. A standard Schirmer test strip was then placed for another 5 min, and the intensity of the staining was compared with a standard color plate. The tear clearance rate was determined by the rate at which the color of the fluorescein dye faded, and graded as 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 or 1/256.¹⁴ The TCR was represented logarithmically.

Keratoepitheliopathy was evaluated by staining the cornea with fluorescein and scoring the area and density of staining. Severity of keratoepitheliopathy was scored by multiplying the area score by the density score, and this product was used as an index of corneal surface damage.¹⁵ The staining area was graded on a numerical scale of 0–3, with 0 representing no punctate staining, 1 representing less than one-third, 2 representing one-third to two-thirds, and 3 representing more than two-thirds staining. The staining density was also graded on a numerical scale of 0–3, with 0 representing no punctate staining, 1 representing sparse density, 2 representing moderate density, and 3 representing high density with overlapping lesions.

To demonstrate the safety of the umbilical cord serum preparation, bacterial and fungal cultures were performed on samples of used and stored sera ($n=8$).

The Wilcoxon signed rank test was used to analyze the data expressed as mean \pm s.d. A $P<0.05$ was considered statistically significant.

Results

Characteristics of patients with severe dry eye associated with GVHD are presented in Table 1. Ten were male patients, and two were female patients. The mean age was 28.5 ± 12.1 years (range, 11–50 years). Pretransplant

diagnoses were acute myelogenous leukemia in six patients, acute lymphocytic leukemia in three patients, chronic myelogenous leukemia in two patients, and non-Hodgkin's lymphoma in one patient. Seven patients underwent bone marrow transplantation, four patients underwent peripheral blood stem cell transplantation, and one patient underwent bone marrow and peripheral blood stem cell transplantation successively. The mean duration between stem cell transplantation and diagnosis of dry eye was 7.3 ± 3.4 months (range, 2–12 months). The mean follow-up period after treatment with umbilical cord serum eyedrops was 7.7 ± 3.9 months (range, 4–17 months).

Changes of symptom, tear film, and ocular surface after umbilical cord serum treatment in patients with dry eye associated with GVHD are shown in Table 2. Two months after treatment, symptom score, corneal sensitivity, tear film BUT, and keratoepitheliopathy score were significantly improved from 3.83 ± 0.38 to 0.92 ± 0.50 ($P<0.01$), from 52.08 ± 6.06 mm to 57.29 ± 3.29 mm ($P=0.01$), from 2.50 ± 0.91 s to 5.67 ± 1.07 s ($P<0.01$), and from 7.42 ± 2.02 to 1.42 ± 0.65 ($P<0.01$), respectively (Figure 1). Six months after treatment, the respective values were 0.83 ± 0.57 , 57.50 ± 3.00 mm, 5.71 ± 1.04 s and 1.29 ± 0.46 ($P<0.01$). There was no significant change in Schirmer test and tear clearance rate after umbilical cord serum treatment. No statistically significant differences existed in the parameters between 2 and 6 months after treatment.

No significant complications associated with the use of umbilical cord serum eyedrops were detected. Bacterial and fungal cultures showed no growth in either used or stored sera.

Discussion

Dry eye is the most frequent ocular complication of chronic GVHD, typically occurs around 6 months after allogeneic hematopoietic stem cell transplantation, and correlates with the severity of GVHD. The primary pathogenesis of dry eye related to GVHD is lacrimal gland dysfunction, and stromal fibroblasts are involved in fibrogenic and immune processes by interacting with T cells in the lacrimal gland.^{16,17} Dry eye associated with GVHD persists after remission of GVHD in most patients and can induce serious ocular complications.¹

Dry eye associated with GVHD is known to be difficult to treat with conventional treatment including the instillation of artificial tears.^{3,9} Murphy *et al.*⁴ reported a patient who responded to 0.05% topical retinoic acid with reversal of conjunctival keratinization and resolution of symptoms. Ogawa *et al.*⁵ treated dry eye successfully in two patients with chronic GVHD with systemic administration of FK506 and corticosteroids, and Kiang *et al.*⁶ used topical cyclosporine A in five patients to control the epithelial keratitis and melting process. Surgical intervention such as multilayer amniotic membrane transplantation may be considered in a GVHD patient with severe dry eye and calcareous corneal degeneration.¹⁸

Serum contains many growth factors and tear components that are important for the proliferation,

Table 1 Characteristics of patients with severe dry eye associated with GVHD

No.	Age (years)	Sex	Original disease	Stem cell Transplantation	Duration between SCT and dry eye (months)	GVHD other than the eyes	Systemic immunosuppression	Conventional ocular treatment	Follow-up after treatment with umbilical cord serum (months)
1	34	M	AML (M5)	BMT	12	Lung	Tacrolimus	Punctal plug, artificial tears	4
2	17	M	AML (M2)	BMT	10	Liver, kidney, skin	Cyclosporin A	Punctal plug, artificial tears, therapeutic contact lens	8
3	19	M	ALL (L1)	BMT	2	Lung, spleen	Tacrolimus, mycophenolate mofetil	Punctal plug, artificial tears, therapeutic contact lens	12
4	50	M	NHL (large B cell)	PBSCT	5	Lung, intestine	Steroid	Punctal plug, artificial tears	17
5	43	M	AML (M3)	BMT	8	Skin, lung	Steroid, cyclosporin A	Punctal plug, artificial tears	4
6	31	M	CML	BMT	6	Lung, spleen, liver	Cyclosporin A	Punctal plug, artificial tears, amniotic membrane transplantation	6
7	11	M	ALL (L1)	BMT/PBSCT	11	Lung	Cyclosporin A	Punctal plug, artificial tears	6
8	19	M	AML (M2)	PBSCT	12	Skin, pancreas	Steroid, cyclosporin A	Punctal plug, artificial tears	4
9	25	F	CML	BMT		Lung, liver	Steroid, cyclosporin A	Punctal plug, artificial tears	9
10	36	M	AML (M3)	PBSCT	5	Lung, skin	Steroid, cyclosporin A	Punctal plug, artificial tears	6
11	18	F	AML (M5)	BMT	3	Kidney	Steroid, cyclosporin A	Punctal plug, artificial tears, therapeutic contact lens	10
12	39	M	ALL (L2)	PBSCT	6	Liver, spleen	Cyclosporin A	Punctal plug, artificial tears	6

Abbreviations: AML = acute myelogenous leukemia; ALL = acute lymphocytic leukemia; BMT = bone marrow transplantation; CML = chronic myelogenous leukemia; GVHD = graft-versus-host-disease; NHL = non-Hodgkin's lymphoma; PBSCT = peripheral blood stem cell transplantation; SCT = stem cell transplantation.

differentiation and maturation of the normal ocular surface epithelium, such as epithelium growth factor (EGF), vitamin A, tumor growth factor- β (TGF- β), acidic and basic fibroblast growth factor (aFGF, bFGF), fibronectin, α_2 macroglobulin, and substance P.⁸ Therefore, autologous serum eyedrops can be applied clinically for the treatment of dry eye including Sjögren's syndrome, persistent epithelial defect, superior limbic keratoconjunctivitis and recurrent corneal erosion.^{8,12} Few studies have been reported on the efficacy and safety of autologous serum eyedrops for the treatment of dry eye associated with GVHD. Rocha *et al.*⁹ reported a beneficial clinical effect of autologous serum application in two cases of GVHD with dry eye. Ogawa *et al.*⁷ also used autologous serum to treat severe dry eye in 14 patients with chronic GVHD. Complaint scores, fluorescein and rose bengal scores, and tear break up time were improved after 4 weeks of serum treatment. However, autologous serum therapy requires repeated blood collection from patients to obtain fresh serum, which may lead to discomfort or treatment refusal. Furthermore, blood sampling is difficult in patients with poor general condition or blood dyscrasia, especially hematologic malignancy.

Umbilical cord serum also contains many growth factors and essential tear components such as epithelial growth factor (EGF), vitamin A and tumor growth factor- β (TGF- β). Especially, EGF and TGF- β concentrations in umbilical cord serum are three and two times higher than those in autologous serum, respectively.¹⁰ Although vitamin A concentration in umbilical cord serum is lower than that in peripheral blood serum, it is much higher than in tears and is sufficient to prevent squamous metaplasia.^{10,12} Recently, several studies have been reported on the beneficial effect of 20% umbilical cord serum eyedrops in the treatment of dry eye syndrome and persistent epithelial defect of the cornea.^{10,11,19} Umbilical cord serum led to faster healing of persistent corneal epithelial defects refractory to medical treatment compared to autologous serum.¹⁹ Compared with autologous serum, umbilical cord serum has several advantages to treat dry eye associated with GVHD.¹⁰ It is not necessary to collect blood in GVHD patients themselves with blood dyscrasia and poor general condition. In addition, because a large amount of serum can be obtained from the umbilical vein at any one time, many patients can benefit from this sampling without waiting for additional preparations.

In the present study, we demonstrated the effect of 20% umbilical cord serum eyedrops for the treatment severe dry eye associated with GVHD. Symptom score, corneal sensitivity, tear film BUT, and keratoepitheliopathy score improved significantly after 2 months of umbilical cord serum treatment, and the improvements were maintained by 6 months after treatment. No significant differences existed in the parameters between 2 and 6 months after treatment. No significant complications associated with the use of umbilical cord serum were observed. Although no growth was found in bacterial and fungal cultures of the serum in this study, the possibility of transmission of blood-borne infectious diseases should be kept in mind. Therefore, additional HIV tests with a shortened window period as well as routine laboratory examination should be

Table 2 Tear film and ocular surface changes after treatment with umbilical cord serum eyedrops in severe dry eye associated with graft-versus-host disease

	Before treatment	2 months after treatment	6 months after treatment	P1	P2	P3
Symptom score	3.83 ± 0.38	0.92 ± 0.50	0.83 ± 0.57	<0.01	<0.01	0.16
Corneal sensitivity (mm)	52.08 ± 6.06	57.29 ± 3.29	57.50 ± 3.00	0.01	<0.01	0.32
Tear film break up time (s)	2.50 ± 0.91	5.67 ± 1.07	5.71 ± 1.04	<0.01	<0.01	0.56
Schirmer test (mm)	2.83 ± 0.38	3.71 ± 2.24	3.54 ± 2.02	0.06	0.11	0.10
Tear clearance rate ((log ₂) ⁻¹)	3.17 ± 0.92	3.42 ± 0.83	3.38 ± 0.77	0.08	0.59	0.71
Keratopathology score	7.42 ± 2.02	1.42 ± 0.65	1.29 ± 0.46	<0.01	<0.01	0.08

Abbreviations: P1 = statistical values between before and 2 months after treatment; P2 = statistical values between before and 6 months after treatment; P3 = statistical values between 2 and 6 months after treatment.

Values are mean ± s.d. (n = 24).

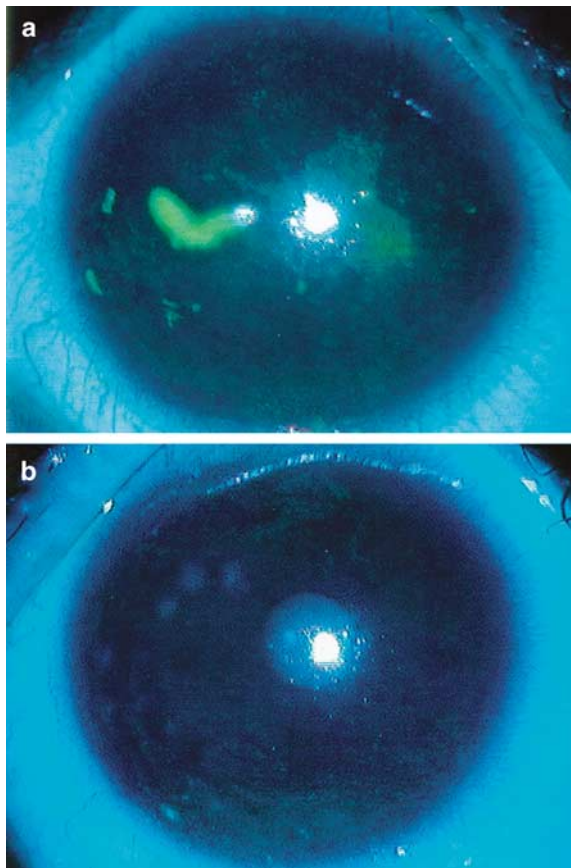


Figure 1 Slit lamp photographs with fluorescein staining in a patient with severe dry eye associated with GVHD. (a) Before umbilical cord serum treatment, the corneal epithelium was eroded and diffusely stained by fluorescein. (b) Two months after treatment of umbilical cord serum eyedrops, the corneal epithelium was markedly improved.

performed in pregnant donors. The limitations of our study are that sample size was small and that no clinical comparison between umbilical cord serum and autologous serum eyedrops was made.

In conclusion, umbilical cord serum eyedrops are safe and may be an effective way to treat severe dry eye associated with GVHD. In the near future, studies with a larger sample size and randomized controlled clinical trials need to be performed.

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