Autologous serum and serum components

Special Issue

Autologous Serum and Serum Components

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Dry eye syndrome is a multifactorial condition on the tear and ocular surface. Autologous serum eye drop is an effective method for treating dry eye. Autologous serum eye drops are now widely used by specialists since a first report in 1975. The results of a systematic study showed that the efficacy of autologous serum eye drops remains ambiguous because its preparation methods and clinical application have not been standardized. To elucidate the efficacy of autologous serum eye drops, well-designed, large-scale, high-quality randomized controlled trials need to be conducted with standardized treatment and use. Since serum components are partially similar to tear components, autologous serum eye drops improve dry eye by supplying tear components such as growth factors, proteins, and vitamins. Adding to the evidence based on the treatment of dry eye, we have found a new treatment candidate from serum: selenoprotein P (SeP). The efficacy of SeP as a treatment for dry eye was revealed by applying SeP eye drops to a dry eye rat model. Compared with phosphate-buffered saline treatment, SeP eye drops significantly reduced the fluorescein score of the cornea and suppressed the oxidative stress in the cornea, which is related to onset of dry eye, leading to improved corneal disorder. We have developed a new dry eye model caused by oxidative stress that will be used to screen candidate molecules for antioxidative activity.

Keywords: autologous serum, serum components, selenium, oxidative stress

Dry eye syndrome is a multifactorial disease of the tears and ocular surface caused by endogenous and environmental (i.e., physical,[1–4] chemical,[5,6] and biological[7]) factors. At present, several effective methods for treating dry eye are known, and autologous serum eye drops are among these. The number of articles on the use of autologous serum as a treatment for ocular surface disease increases each year (Fig. 1). A search of PubMed in November 2017 retrieved 134 reports on "autologous serum 'eye drops'" and 119 reports on "autologous serum 'dry eye'." Of these 134 reports, 44 that were published in 2015 to 2017 are summarized in Table 1.[8–51] Twelve were review articles, 11 were basic research articles, and the remainder were clinical studies (Table 1). The first report on autologous serum for the treatment of ocular surface diseases was published by Ralph et al.[52] in 1975. They continuously delivered a serum or drug solution to the ocular surface of patients with ocular surface disease using a mobile ocular perfusion pump, and serum treatment was shown to be partially effective. The efficacy of autologous serum eye drops for the treatment of dry eye was first mentioned by Fox et al.[53] in 1984. Many studies from various countries on the efficacy of autologous serum were subsequently reported. In Japan, studies on autologous serum eye drops were conducted by Tsubota's group[54–56] and Takamura et al.,[57] who showed the usefulness of autologous serum eye drops in the treatment of dry eye caused by several diseases.[54–57] At present, autologous serum eye drops are globally used as a therapeutic intervention for dry eye.

Autologous Serum Eye Drops and Other Therapeutic Interventions for Dry Eye Syndrome

Sy et al.[38] conducted an international survey to identify the most common treatments for dry eye used by specialists. The most commonly prescribed topical treatments for aqueous-deficient dry eye included 0.05% cyclosporine A (68%), 0.1% fluorometholone (60%), 0.5% loteprednol etabonate (51%), and autologous serum eye drops[33] (49%), and nontopical medications included essential fatty acid supplements (69%), low-dose doxycycline (oral; 61%), and flaxseed supplements (33%), as well as punctal plugs[33] (75%). The top three signs and symptoms that were indicators of treatment response were fluorescein staining of the cornea, reduced foreign body sensation, and reduced burning sensation.

In Australia, the Red Cross Blood Service manufactures and distributes autologous serum eye drops to eligible patients with dry eye, persistent corneal epithelial defects,

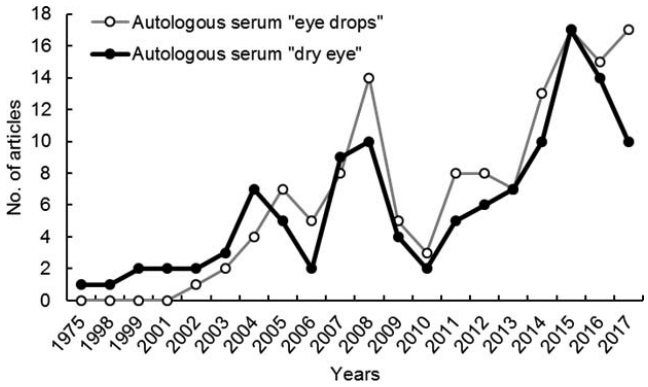

Figure 1. The number of published articles, by year, retrieved from a PubMed search conducted in November 2017 using the terms "autologous serum 'eye drops'" (*open circle*) and "autologous serum 'dry eye'" (*closed circle*). The vertical axis shows the number of articles.

Table 1. Articles on Autologous Serum Eye Drops Published in 2015 Through 2017 (October). The Articles Were Searched in PubMed Using the Keyword "Autologous Serum 'Eye Drops'"

Year	Authors	Journal	Article Type	Ref. No.
2017	Giannaccare G, et al.	*Transfus Apher Sci*. 2017;56:595–604.	Review	8
2017	Alio JL, et al.	*Ophthalmol Ther*. 2017;6:1–9.	Clinical study	9
2017	Wu MF, et al.	*Int J Ophthalmol*. 2017;10:908–913.	Basic research	10
2017	Akcam HT, et al.	*Clin Exp Optom*. 2017;101:34–37.	Clinical study	11
2017	Sanchez-Avila RM, et al.	*Int Ophthalmol*. 2018;38:1193–1204.	Clinical study	12
2017	Marks DC, et al.	*Vox Sang*. 2017;112:310–317.	Basic research	13
2017	Jian-Wei L, et al.	*Cutan Ocul Toxicol*. 2017;36:377–380.	Clinical study	14
2017	Etxebarria J, et al.	*Acta Ophthalmol*. 2017;95:e693–e705.	Basic research	15
2017	Pan Q, et al.	*Cochrane Database Syst Rev*. 2017;2:CD009327.	Review	16
2017	Stachon T, et al.	*Klin Monbl Augenheilkd*. 2017;234:1015–1018.	Basic research	17
2017	Mahelková G, et al.	*Clin Exp Optom*. 2017;100:243–249.	Clinical study	18
2017	Piccin A, et al.	*Blood Transfus*. 2017;15:333–340.	Review	19
2017	Azari AA, et al.	*Cutan Ocul Toxicol*. 2017;36:152–156.	Clinical study	20
2017	Tahmaz V, et al.	*Br J Ophthalmol*. 2017;101:322–326.	Clinical study	21
2017	Wang WY, et al.	*Eye Contact Lens*. 2017;43:225–229.	Clinical study	22
2016	Semeraro F, et al.	*In Vivo*. 2016;30:931–938.	Clinical study	23
2016	Asena L, et al.	*Curr Eye Res*. 2017;42:506–512.	Basic research	24
2016	Gus PI, et al.	*Oxid Med Cell Longev*. 2016;2016:9780193.	Clinical study	25
2016	Anitua E, et al.	*Exp Eye Res*. 2016;151:115–121.	Basic research	26
2016	Lee YK, et al.	*Cont Lens Anterior Eye*. 2016;39:425–430.	Clinical study	27
2016	Tseng CL, et al.	*PLoS One*. 2016;11:e0153573.	Basic research	28
2016	Lee JH, et al.	*Korean J Ophthalmol*. 2016;30:101–107.	Clinical trial	29
2016	von Hofsten J, et al.	*Int Med Case Rep J*. 2016;9:47–54.	Clinical study	30
2016	Rybickova I, et al.	*Biomed Pap*. 2016;160:271–275.	Clinical study	31
2016	van der Meer PF, et al.	*Transfus Apher Sci*. 2016;54:164–167.	Review	32
2016	Marshall LL, et al.	*Consult Pharm*. 2016;31:96–106.	Review	33
2016	López-García JS, et al.	*Cornea*. 2016;35:336–341.	Clinical study	34
2016	Hondur AM, et al.	*Curr Eye Res*. 2016;41:15–19.	Clinical study	35
2015	Katsakoulas I, et al.	*Int J Pharm Compd*. 2015;19:252–260.	Basic research	36
2015	Mondy P, et al.	*Transfus Apher Sci*. 2015;53:404–411.	Review	37
2015	Sy A, et al.	*BMC Ophthalmol*. 2015;15:133.	Basic research	38
2015	Mahelková G, et al.	*Cesk Slov Oftalmol*. 2015;71:184–188.	Clinical study	39
2015	Liu Y, et al.	*Cornea*. 2015;34:1214–1220.	Clinical study	40
2015	Stenwall PA, et al.	*Acta Ophthalmol*. 2015;93:654–657.	Clinical study	41
2015	Li J, et al.	*Cornea*. 2015;34:1072–1078.	Clinical trial	42
2015	van der Meer PF, et al.	*Transfus Apher Sci*. 2015;53:99–100.	Review	43
2015	Marks DC, et al.	*Transfus Apher Sci*. 2015;53:92–94.	Review	44
2015	Lin SJ, et al.	*Taiwan J Ophthalmol*. 2015;5:109–113.	Clinical study	45
2015	Wirostko B, et al.	*Ocul Surf*. 2015;13:204–212.	Review	46
2015	Espinosa A, et al.	*Transfus Apher Sci*. 2015;53:88–91.	Review	47
2015	Anitua E, et al.	*Acta Ophthalmol*. 2015;93:e605–e614.	Review	48
2015	Anitua E, et al.	*Exp Eye Res*. 2015;135:118–126.	Basic research	49
2015	Azari AA, et al.	*Eye Contact Lens*. 2015;41:133–140.	Review	50
2015	Blasetti F, et al.	*J Infect Dev Ctries*. 2015;9:55–59.	Basic research	51

and nonhealing corneal ulcers.[44] Since 2005, the Blood Service has collected whole blood from patients for the manufacturing of serum eye drops using a national, standardized process to produce 20% of serum drops. Demand for serum eye drops in Australia is increasing every year, with a 30% increase in 12 months during 2014 to 2015.[44] Based on the results of these studies, it can be concluded that autologous serum eye drops are a well-established treatment for dry eye disease.

Clinical Studies of Autologous Serum Eye Drops

A small cohort study on patients with mixed ocular surface disease treated with autologous serum eye drops was conducted at a Swedish tertiary referral center.[30] All cases ($n = 32$ eyes, 24 patients) were treated with autologous serum eye drops. The results of the study showed that patients with a persistent epithelial defect or superficial punctate keratitis responded well to treatment with autologous serum eye drops. However, large-scale randomized clinical trials are needed to fully comprehend the role of the eyes drops in the treatment of ocular surface disease.

Pan et al.[16,58] reported two systematic reviews in Cochrane Database of Systematic Reviews. In a more recent report,[16] they conducted a review to evaluate the efficacy and safety of autologous serum eye drops for adults with dry eye administered alone or in combination with artificial tears compared with artificial tears alone, saline, placebo, or no treatment. The results showed that in 20% of patients, autologous serum eye drops provided some benefits in improving patient-reported

Table 2. Comparison of Tear and Serum Components

Components	Concentration	
	Tear, Basal	Serum†
Proteins		
Total protein	7.51 mg/mL	66–81 mg/mL
Lysozyme	2.36 mg/mL	5.0–10.2 μg/mL
Lactoferrin	1.84 mg/mL	0.17–0.28 mg/mL
Albumin	1.3 mg/mL	41–51 mg/mL
IgA	0.30 mg/mL	0.93–3.93 mg/mL
IgD	ND	0.03 mg/mL
IgE	0.1 μg/mL	0.4 μg/mL
IgG	0.126 mg/L	8.61–17.47 mg/mL
IgM	0.86 μg/mL	0.33–1.83 mg/mL
CuZn-SOD	103 ng/mg protein	ND‡
Growth factors		
EGF	1.66 ng/mL	0.72 ng/mL
TGF-α, male	247 pg/mL	147 pg/mL
TGF-α, female	180 pg/mL	147 pg/mL
TGF-β1	Not detected	140.3 ng/mL
TGF-β1*	2.32 ng/mL	–
TGF-β2	55 pg/mL	–
Vitamins		
Vitamin A	16 ng/mL	200–500 ng/mL
Vitamin C	117 μg/mL	5–9 μg/mL
Antioxidants		
Tyrosine	45 μM	77 μM
Glutathione	107 μM	ND‡
Carbohydrate		
Glucose	26 mg/L	0.6–1.2 g/L
Electrolytes		
Na^+	148.5 mM	138–145 mM
K^+	18.7 mM	3.6–4.8 mM
Ca^{++}	1.73 mM	8.8–10.1 mM
Cl^-	112 mM	101–108 mM
HCO_3–	26 mM	21–29 mM
NO_3–	0.14 mM	0.19 mM
PO_43–	0.22 mM	1.42 mM
SO_42–	0.39 mM	0.53 mM

* Acid-activated tear.

† Each value is of normal concentration in the serum.

‡ These components are present in red blood cells at high concentration. See Refs. 46 through 51.

symptoms in the short term (2 weeks), but longer periods of follow-up indicated no evidence of long-term improvement. Objective clinical measures of the ocular surface showed no clear effect. The authors concluded that well-designed, large-scale, high-quality randomized controlled trials are needed to clarify the efficacy of autologous serum eye drops for the treatment of dry eye.

Despite this, autologous serum eye drops have been found to have some value in the treatment of dry eye. To elucidate the efficacy of the eye drops, the methods for its preparation, manufacturing, and storage (such as clotting time, centrifugation condition, dilution ratio, and the most suitable diluent) must be well established, and its clinical application for dry eye patients should be optimized.

Components of Serum and Tears

Autologous serum eye drops are thought to alleviate dry eye by supplying tear components, such as various growth factors, vitamins, and proteins, that aid in the maintenance of the ocular surface. Table 2 contains a comparison of tear[59–62] and serum components.[59,63,64] The concentration of total tear protein is 7.51 mg/mL, which is approximately 10% lower than that of serum. Lysozyme, lactoferrin, and albumin are the major proteins in tears, whereas albumin and IgG are the major proteins in serum. Since lysozyme protects against infection on the ocular surface via antimicrobial activity and the lactoferrin transports iron to the cornea, those proteins are thought to be secreted at high concentration in tears. Autologous serum eye drops are prepared using autologous serum under sterile conditions (Fig. 2). Blood collected from a patient is clotted and centrifuged to prepare the serum. The prepared serum is diluted to 20% to 100% with saline and preserved in a refrigerator or freezer in case of the need for long-term preservation. Because infection via the serum can occur, it is necessary to prepare autologous serum eye drops for every patient to prevent infection. Another problem is that serum is physically unstable and probably degraded by serum internal enzymes. Use of serum components for eye drops, such as growth factors, albumin, and lactoferrin, could minimize these problems. Studies with a focus on the efficacy of serum components for the treatment of dry eye have been increasing since about 2007.

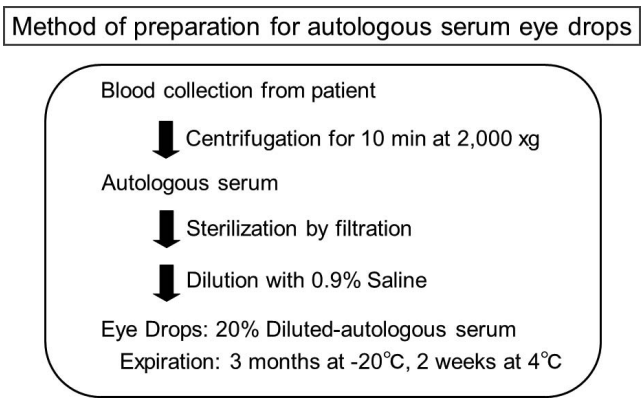

Figure 2. Method of preparation for autologous serum eye drops. Autologous serum eye drops were prepared using autoserum collected from each patient in sterile conditions.

Growth Factors

Tears contain several growth factors that are also contained in serum (Table 2). We previously reported an in vitro experiment using human conjunctival epithelial cell lines (CCLs) that demonstrated that epidermal growth factors (EGFs) and retinoic acid play key roles in the maintenance of the ocular surface.[65] In an animal dry eye model experiment, EGF eye drops improved tear breakup time (BUT) and fluorescein staining, but not inflammatory index and tear volume.[66] The authors concluded that EGF has potential as a therapeutic agent in the clinical treatment of dry eye.[66] Lou-Bonafonte et al.[67] reported a systematic review on clinical studies on the posology, efficacy, and safety of EGF eye drops in the treatment of certain human corneal disorders. EGF eye drops were found to be approximately 50 times more effective than vehicle eye drops in achieving accelerated and successful corneal wound healing. EGF eye drops (50–1000 ng, two or three times/day) could be a useful treatment for promoting postoperative refractive surgery and reversing cases of keratopathy secondary to systematic EGF receptor inhibitors, diabetic keratopathy, and other corneal and conjunctival disorders.

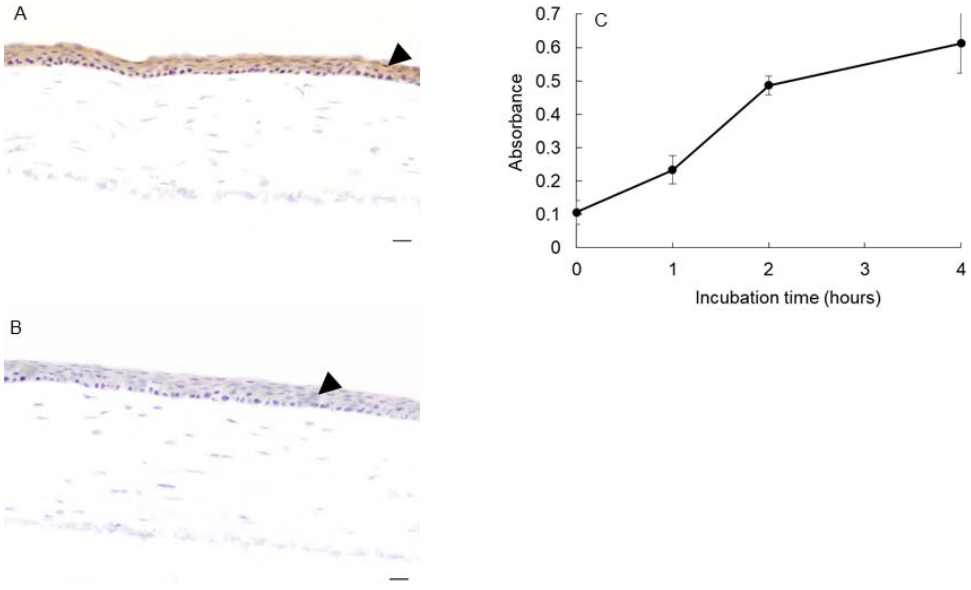

FIGURE 3. Lactoferrin uptake into corneal epithelial cells. (**A**, **B**) Immunohistochemical staining of lactoferrin receptor LRP1 in a rat cornea was performed using anti-LRP1 rabbit antibody (**A**) and normal rabbit IgG (**B**). *Scale bar*: 20 μm. Original magnification: ×20. (**C**) Lactoferrin uptake into corneal epithelial cells. The cells were incubated in the presence of 500 ng/mL lactoferrin for 0, 1, 2, or 4 hours. Results are expressed as mean ± standard deviation ($n = 6$). Reprinted with permission from Higuchi A, Inoue H, Kaneko Y, Oonishi E, Tsubota K. Selenium-binding lactoferrin is taken into corneal epithelial cells by a receptor and prevents corneal damage in dry eye model animals. *Sci Rep*. 2016;6:36903. © 2016 Higuchi et al. This is an open access article distributed under the terms of the Creative Commons Attribution License.

The efficacy of growth factor eye drops has been supported by clinical studies that have used eye drops prepared from umbilical cord blood serum (UCBS)[8] or platelet-rich plasma (PLP).[9] UCBS contains more growth factors than other blood-derived preparations, with higher levels of EGF, TGF-β, NGF, and VEGF.[68] Platelets are blood-derived components fundamental to the wound healing process that deliver many growth factors contained in their α-granules, such as platelet-derived growth factor (PDGF) and TGF-β. UCBS eye drops were more effective than autologous serum in the treatment of severe dry eye, particularly when secondary to graft-versus-host disease[69] and Sjögren's syndrome.[8,70] Autologous PLP eye drops were an effective treatment in improving the signs and symptoms of patients suffering from moderate-to-severe chronic dry eye disease.[8,9,71,72]

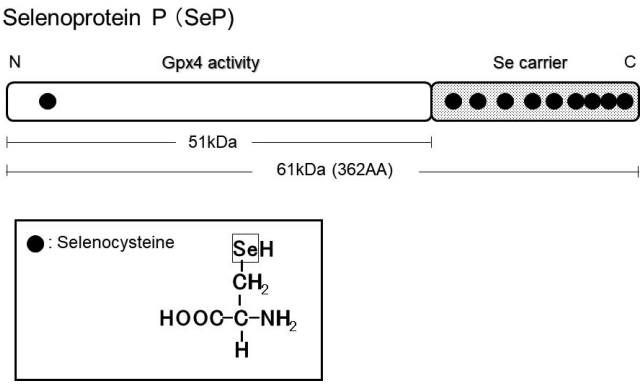

FIGURE 4. Schematic of selenoprotein P (SeP). SeP is a selenium (Se)-transfer plasma glycoprotein that contains 10 selenocysteine (Sec) residues. One of the Secs is located in the N-terminal, and the other nine residues are localized in the C-terminal. Sec residues in the C-terminal function as a supplier of Se to the cells. *In a square frame*: structural formula Sec. Sec, the 21st amino acid, is a cysteine analogue with a sulfur atom replaced by a selenium atom. Twenty-five selenoprotein genes are present in the human genome, such as glutathione peroxidase (GPx) and thioredoxin reductase (TR). GPx and TR are implicated in the regulation of oxidative stress in cells. Since Sec is located in the active site of GPx, Sec and selenium are essential for GPx activity.

Growth factors promote the proliferation and differentiation of ocular surface cells. Loo et al.[73] showed EGF-induced bcl-2 expression in serum-free mouse embryo cell lines. One of the mechanisms of the effectiveness of EGF relates to the antiapoptotic effect caused by bcl-2 induction. In the author's opinion, the efficacy of growth factors in the treatment of dry eye is at least partially responsible for maintaining cellular homeostasis.

VITAMINS

Vitamins are likewise found in tears and serum (Table 2). Vitamin A is important in maintaining the health of the ocular surface, affecting cellular regulation and differentiation, and keratomalacia occurs when the diet is grossly deficient in vitamin A.[74] Vitamin A deficiency adversely affects epithelial cells in the eyelid, conjunctiva, and cornea. Hence, an absence of vitamin A causes loss of goblet cells and leads to increased epidermal keratinization and squamous metaplasia of the mucous membranes, which generally includes the cornea and conjunctiva.[75] In previous clinical studies, retinoic acid has been shown to be useful in the treatment of dry eye[76,77] and Stevens–Johnson syndrome.[78] Kim et al.[79] conducted a comparison of the efficacy of vitamin A and 0.05% cyclosporine A eye drops in treating patients with dry eye and showed that both eye drops were effective. Vitamins A, C, and E have antioxidative properties. Antioxidative vitamins protect the cornea, lens, and retina against oxidative stress and maintain the functions of those tissues.[74] However, there have not been any remarkable reports on the efficacy of vitamin C eye drops in the treatment of dry eye.

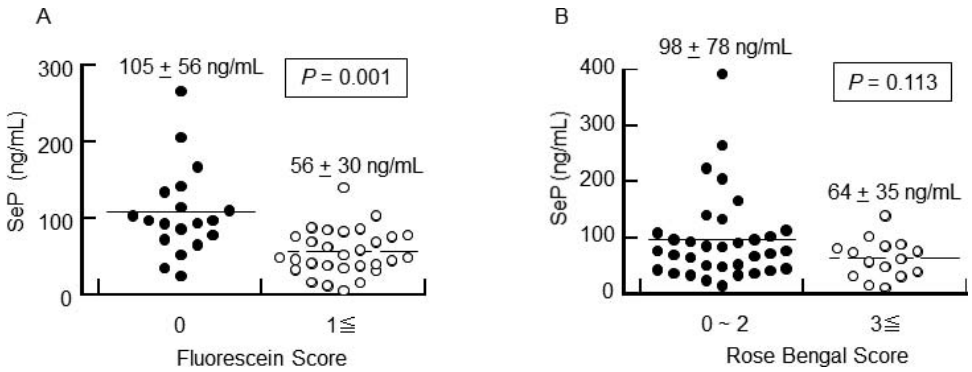

Figure 5. Concentration of selenoprotein P (SeP) in patients' tears. SeP concentration with or without corneal disorder was measured. Scores are indices of dry eye (**A**, fluorescein scores of ≥1; **B**, rose bengal score of ≥3). Reprinted with permission from Higuchi A, Takahashi K, Hirashima M, Kawakita T, Tsubota K. Selenoprotein P controls oxidative stress in cornea. *PLoS One*. 2010;5:e9911. © 2010 Higuchi et al. This is an open access article distributed under the terms of the Creative Commons Attribution License.

Albumin

Albumin is a principal protein that is plentiful in tears and serum (Table 2). Human serum albumin (HSA) is a monomeric single-chain protein constituted from 585 amino acids that does not have any prosthetic group. HSA is a remarkably stable protein that is widely distributed in body fluids. It is a multifunctional carrier protein, and a number of albumin-binding proteins have been identified from the endothelium in various tissues.[80,81] Our clinical pilot study[82] showed that 5% HSA eye drops for 4 weeks statistically significantly improved fluorescein staining, rose bengal staining, BUT, and symptomatic face score. An in vitro experiment using CCL and human corneal epithelial (CEPI) cell lines showed that HSA suppressed apoptosis, accompanied by the inhibition of caspase-3 activity.[83]

Recently, to elucidate the efficacy of albumin eye drops in the treatment of severe ocular surface defects, a case–control study that used 5% albumin and 0.1% hyaluronan eye drops was performed.[84] Although healing times for both treatments were similar, albumin eye drops seemed to reduce the recurrence of sterile corneal ulcers. The authors concluded that the efficacy of both treatments was comparable, but that additional large-scale studies were necessary.

Lactoferrin

Lactoferrin is an iron-binding glycoprotein that is found in most exocrine fluids, such as saliva, bile, pancreatic fluid, amniotic fluid, and tears.[85] The most common metal ion associated with lactoferrin in vivo is iron in its ferric (III) form. Lactoferrin can also bind to other metal ions, such as copper and magnesium. Tear fluid contains many kinds of antioxidative stress compounds, and lactoferrin is one of these, which protects the corneal epithelium against UV irradiation.[86] Previous studies have demonstrated that the concentration of lacrimal lactoferrin reduced[87] and oral administration of lactoferrin improved the symptoms of dry eye patients with Sjögren's syndrome.[88] Using a rabbit dry eye model, Fujiwara et al.[89] showed that lactoferrin eye drops rescued corneal damage and that this effect was more remarkable using 1% apolactoferrin not saturated with iron, compared with iron-saturated lactoferrin.[89] However, in our recent study that used a dry eye model rat caused by the removal of the lacrimal glands, 1% apolactoferrin eye drops were found to be only slightly effective.[90] Apolactoferrin eye drops at a high dose (5%) showed a weak, and not statistically significant, improvement in corneal damage. Since lactoferrin is a physiological iron carrier to the corneal epithelium, it is hypothesized that a

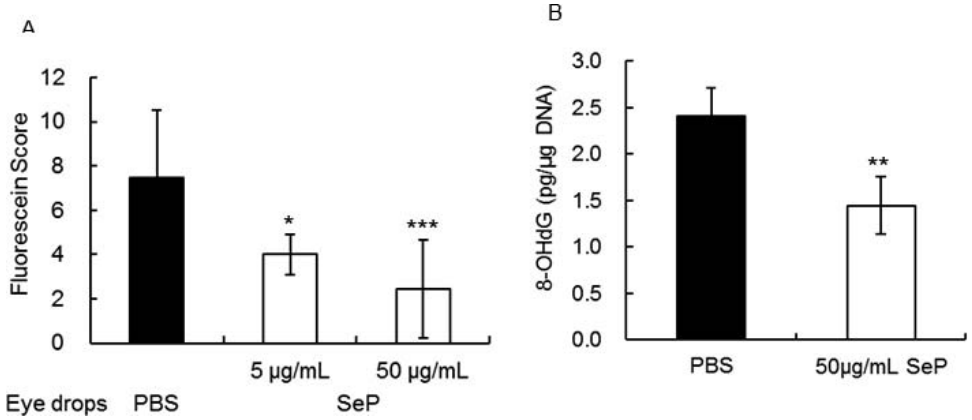

Figure 6. Evaluation of selenoprotein P (SeP) eye drops using dry eye model rats. (**A**) Fluorescein score of a dry eye model rat treated with PBS or SeP eye drops. Results are expressed as mean ± standard deviation ($n = 10$). Dunnett's test was used to determine the significance of differences. * and *** Indicate significant differences from the PBS treatment result ($P < 0.05$ and < 0.005, respectively). (**B**) Ratio of 8-OHdG content in the corneas of dry eye model rats between PBS and SeP eye drops. Results are expressed as mean ± standard deviation ($n = 10$). *t*-test was used to determine the significance of the differences. ** Indicates a significant difference from the PBS treatment result, $P < 0.05$. Reprinted with permission from Higuchi A, Takahashi K, Hirashima M, Kawakita T, Tsubota K. Selenoprotein P controls oxidative stress in cornea. *PLoS One*. 2010;5:e9911. © 2010 Higuchi et al. This is an open access article distributed under the terms of the Creative Commons Attribution License.

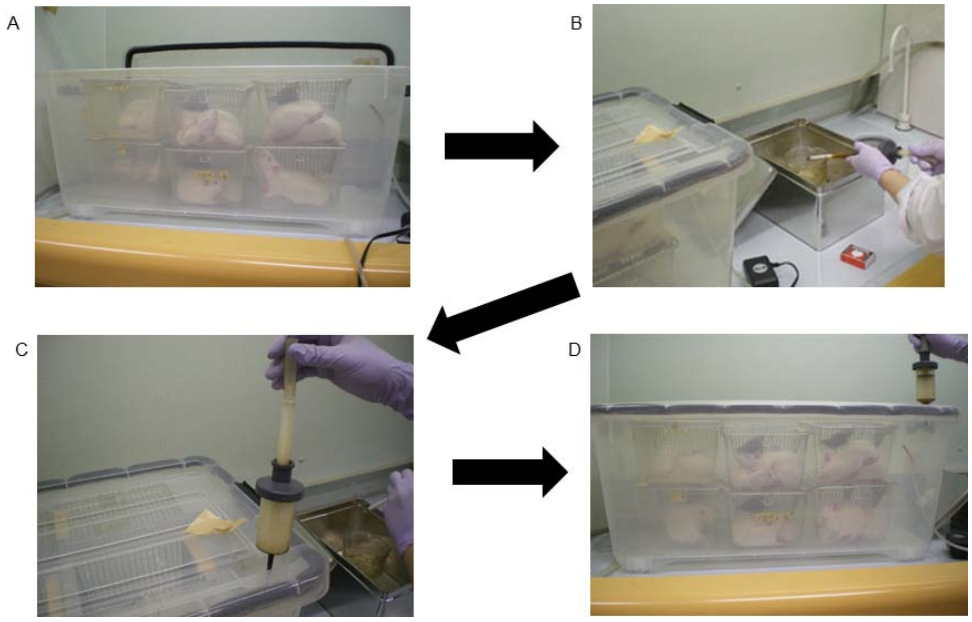

FIGURE 7. Tobacco smoke exposure rat model, prepared as described in our previous studies.[55,64] The rat was placed in an experimental smoking chamber (60 × 40 × 35 cm) (**A**) with continuous fresh air ventilation for 3 hours per day for 5 days. Three hundred milliliters of mainstream cigarette smoke prepared from cigarettes containing 14 mg tar (Seven Stars; Japan Tobacco, Tokyo, Japan) was injected into the smoking chamber (**B**, **C**) every 30 minutes for a total of six times over a 3-hour period per day for 5 days during the exposure period (**D**).

physiological mechanism, for example, a ligand-uptake receptor system, is essential for iron uptake to the corneal epithelium. Recently, we reported that a lactoferrin uptake receptor, LRP-1, was expressed in the corneal epithelium (Figs. 3A, 3B) and that lactoferrin was taken up into the CEPI cells (Fig. 3C). Lactoferrin was promptly taken up into the CEPI cells via the lactoferrin receptor.[91]

Selenoprotein P

Although the above-mentioned serum components have been found to be useful in the treatment of dry eye, there are no candidates with excellent efficacy. We tried to find a new candidate from serum for the treatment of dry eye.[92] Human serum was fractionated using column chromatography, and the index of purification of the candidate was cellular viability. The details can be found in Higuchi et al.[92] The fraction finally obtained was separated using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and the candidate band of SDS-PAGE was cut out for identification using the Edman method. We finally identified selenoprotein P (SeP) as a new candidate for the treatment of dry eye. Figure 4 shows a schematic of SeP.

SeP is a selenium-transfer plasma glycoprotein[93] that is present in extracellular fluids such as plasma[94] and milk.[95] The principal source of SeP is thought to be the liver.[96] SeP is a bifunctional protein,[97] as the Sec residue in the N-terminal shows GPx activity[98] and Sec residues in the C-terminal function as a cellular selenium supplier.[99] Selenium is an essential trace element for the human body and performs physiological functions as a selenocysteine (Fig. 4), which regulate oxidative stress in the cells. We have reported that SeP is synthesized in the lacrimal gland and secreted in tears to supply selenium to the cornea.[92] Human tears (2 μL) were obtained by capillary collection from patients ($n = 49$) with or without dry eye symptoms. The concentration of SeP in patients without corneal disorder was significantly higher than that in patients with corneal disorder ($P = 0.01$), as evaluated using fluorescein scoring (Fig. 5A), and a similar result was obtained using rose bengal scoring (Fig. 5B). The efficacy of SeP in the treatment of dry eye was evaluated by applying SeP eye drops to a dry eye rat model. The fluorescein scores of corneas treated with SeP eye drops were significantly lower than in those treated with PBS (Fig. 6A). SeP eye drops suppressed the production of the oxidative stress marker 8-OHdG in the cornea (Fig. 6B). SeP eye drops improved corneal disorder associated with the suppression of oxidative stress in the cornea of a dry eye model rat.

Oxidative Stress and Dry Eye

Ocular surface is strongly affected by oxidative stress caused by several factors, such as light exposure (including ultraviolet irradiation), molecular oxygen, chemical compounds, and direct contact with airflow. Oxidative stress causes and/or exacerbates many ocular diseases, including dry eye. Serum and tears contain antioxidative components: SeP, lactoferrin, glutathione, and vitamins (Table 2). To evaluate the efficacy of antioxidative components in the treatment of dry eye, we attempted to prepare a new dry eye model principally caused by oxidative stress. Since smoking elevates oxidative stress in the whole body, we placed rats in the experimental smoking chamber and systemically exposed them to the main tobacco smoke, that is, a rat tobacco smoke exposure model[100] (Fig. 7). We plan to use the tobacco smoke exposure dry eye model to screen candidate molecules for antioxidative effects.

Conclusions

At the present time, autologous serum eye drops are a widely used treatment for dry eye syndrome both in Japan and internationally. However, there are several problems relating to the methods used for the preparation and preservation of

autologous serum that need to be addressed. Furthermore, research on autologous serum eye drops will lead to the development of new drugs for the treatment of dry eye based on compounds derived from serum components.

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