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Original article

Ocular moisturizing effects of rooster comb degradation product containing low-molecular hyaluronic acid and collagen peptides (CRISTA®) in individuals with dry eye-related symptoms: A randomized, double-blind, placebo-controlled, parallel-group study

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Abstract

***Purpose*:** A double-blind randomized controlled clinical trial with parallel-group comparison was conducted to confirm the alternations in subjective and objective symptoms, and the safety of ingestion of a test food product (CRISTA®), which contains a high concentration of rooster comb enzymatic degradation product. The primary components are low molecular weight hyaluronic acid (HA) and collagen peptide. Effectiveness on ocular moisturizing effects and safety were assessed.

***Methods*:** Thirty healthy subjects who were experienced dry or eye fatigue on a daily basis due to viewing an electronic display for work, were randomly classified into two groups, the test food product group and the control (placebo) group. Among them, excluding one participant (the test food product group), who met exclusion criteria, twenty-nine subjects were subject of analysis for validity verification assessment items: fourteen food product group participants (male: seven, female: seven, age: 38.2 ± 3.2 years) and control food group participants (male: eight, female: seven, age: 38.4 ± 2.7 years).

***Results*:** After four-week ingestion of test product, significant increase of lacrimation was confirmed with Schirmer's test. Significant differences were shown in both the test product group and the control group. The effectiveness of the test product was verified to keep the eyes moisturized. There were no differences between two groups regarding Dry Eye Quality of Life (QOL) Questionnaire, Visual Analog Scale for subjective symptoms, Anti-aging QOL Common Questionnaire, tear break-up time, visual acuity, and intra-ocular pressure. The safety of the continuous oral ingestion for as long as four weeks was verified.

***Conclusion*:** This test food product is promising as an effective and safe oral ingestion functional food to prevent eye dryness.

***KEY WORDS*:** randomized controlled clinical comparative trial, low molecular weight hyaluronic acid, dry eye, Schirmer's test, tear break-up time

Introduction

The intercellular matrix is a network of diverse molecular structures existing in the outer space of parenchymal cells. Intercellular matrix in humans contains glycoproteins such as collagen, proteoglycan, fibronectin, and laminin, and also mucopolysaccharides such as hyaluronic acid and chondroitin sulfa. Quantitative and qualitative deteriorations of intercellular matrix are intensely related to the onset and progression of symptoms of skin aging and osteoarthritis [1].

The Society for Glycative Stress Research have been committed to studies for people who suffer from knee joint and lower back pains. An uncontrolled trial was conducted for the ingestion of the test food product (INJUV®), which contained a rooster comb enzymatic degradation product and the main components were low molecular weight hyaluronic acid (HA) and collagen peptide, to examine alternations of subjective and objective symptoms. The trial confirmed the improvements in the knee joint and lower back pains and the effectiveness on enlargements of articulatio genus. Moreover, improvements of asthenopia were observed [2]. Thus, an uncontrolled trial was conducted for individuals who were aware of eye dryness or eye strain, because tears contain HA, which has protective function against ocular dryness

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and dry eye. This trial confirmed significant improvement in tear break-up time and significant decrease in intra-ocular pressure, suggesting a possibility of effectiveness on ocular dryness [3].

People have been increasingly exposed to risk factors for eye health conditions in modern society due to long work hours in front of personal computers, dryness due to the use of air conditioners, and wearing contact lenses. An increasing number of patients complain of dry eye syndrome, feelings of dryness, eye pains, hazy and cloudy vision, and blurred vision. According to Osaka Study in 2013, 79.5% of females and 60.2% of males are diagnosed with dry eye syndrome [4]. Since the Covid-19 pandemic, patients with dry eye syndrome have increased since 2020 [5]. These exacerbated medical conditions could remarkably reduce quality of life. Leaving damage without clinical treatments would lead to the aggravation of diseases. Consequently, cornea and conjunctiva are injured, and disordered physical conditions could occur such as headache and dizziness or vertigo. Therefore, when eye conditions are deteriorated, immediate treatments are essentially required to improve symptoms.

It is common for the improvement of eye conditions to apply eyedrops directly to eyes such as artificial tears, HA drug formulation, medical agent promoting the formation and secretion of mucin, and eyedrops containing anti-inflammatory drug, such as an adrenocortical steroid (Patent Literature: unexamined patent application 201938783 patent publication 1). The applications of these eye drops, ophthalmic solutions, could be burdensome. Instructions on how to apply eye drops are as follows: Everytime wash your hands before handling your eye drops or touching your eyes. Tilt your head back and pull your lower eyelid down. Don't let the bottle touch to your eye, eyelid, and eyelashes. Squeeze the bottle gently and let one drip of eye drop fall into the conjunctival sac. Close your eyes lightly. Wipe out excessive eyedrops from edge of eyes and skin using facial tissues. The directions for usage are bothersome. In addition, you have to be cautious of storage methods, expiration date, and allergic reactions due to preservative. Therefore, the development of a desirable oral ingestion is expected. It would be simple and easy to ingest and store to effectively improve eye conditions. However, there are few proven functional components via oral ingestion to effectively improve ocular problems.

Considering these backgrounds, we conducted the double-blind randomized placebo-controlled clinical trial with comparison of parallel-group design, to verify the test food product (CRISTA®), which is condensed in a high concentration from INJUV®.

Subjects and method

1. Implementation structure of the trial

GlcCer and Cer (***Fig. 1***) were purified according to our previ The present study was conducted, for the human rights and safety of research participants and the data trust of the trial, after obtaining the examination and approval of the

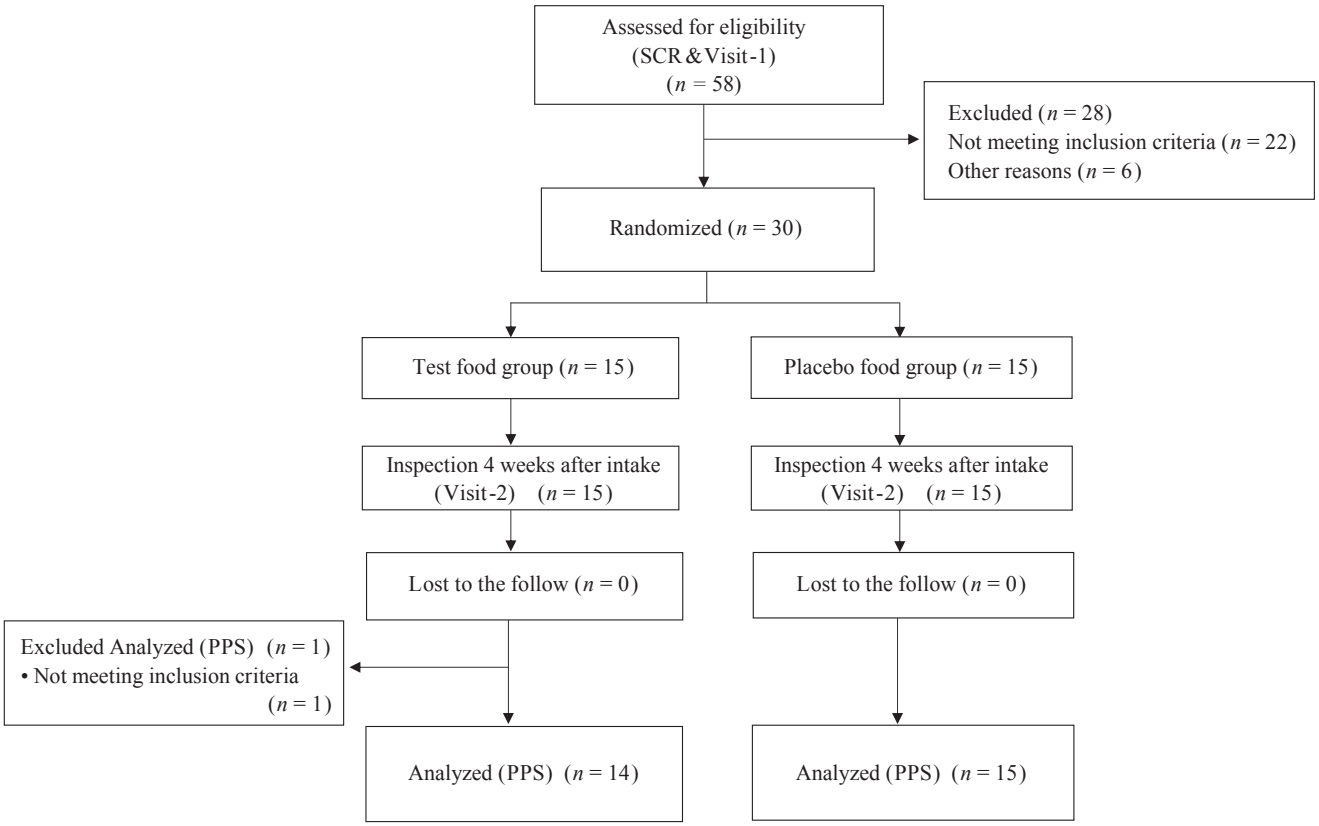

Fig. 1. **Flow of analysis from subject enrollment.**

PPS, Per Protocol Set.

Ethical Committee of the third party, general incorporated association Society for Glycative Stress Research, regarding "Research involving human subjects" (approval number: 2022-008, December 21st, 2022). The trial was performed by Ueno Asagao Clinic (principal investigator of the trial: the president of the hospital, Atsushi Nakajima), and Hongo san-chome eye clinic (Sub investigator: the president of the hospital, Kayako Shibuya) based on the clinical trial protocol (UMIN trial ID: UMIN000049882). The present trial was conducted in compliance with ethical principles based on the Declaration of Helsinki (Amendment, 2013 WMA Fortaleza "Brazil") and Ethical Guideline for Medical and Biological Research principles involving human subjects (Implemented in 2021, Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry. Notification: No.1).

2. Research Participants

In the recruitment of research participants, target participants were provided with explanation of the trial, and 58 candidates who satisfied the inclusion criteria underwent prior inspections. Thirty participants were selected for the final examination, among candidates who investigator and subinvestigators decided as appropriate participants based on the selection standard, satisfying the selection criteria and did not match the exclusion criteria. The number of cases was decided and set up as follows: In a trial with comparison between groups, thirteen participates per group were required such as comparisons in the primary endpoint between groups and comparisons before- and after-ingestion to perform statistical tests and estimations. With the consideration of risks for social circumstances and discontinuation/dropout, the necessary number of cases was decided as fifteen cases per group.

Key inclusion criteria for subjects as follows:

1) Men and women between 20 and 60 years of age at the time of obtaining consent to participate in the study.
2) Healthy subjects with no chronic physical diseases, including ocular or skin diseases.
3) Subjects who are aware of dry eye-like symptoms in daily life (eye fatigue, eye irritation, eye dryness, eye discomfort, eye pain, red eyes, difficulty opening eyes in the morning, burning or itchy eyes).
4) Subjects who perform VDT (visual display terminals) work in their daily lives for 20 hours or more per week, 5 days per week (including the time spent playing video games and operating computers and cell phones).
5) Subjects must have best corrected visual acuity of 1.0 or better in both eyes and not wear contact lenses or be able to change them to eyeglasses during the examination period.
6) Subjects who have been fully informed of the purpose and content of this examination, have the ability to consent, understand it well, volunteer to participate, and agree to participate in this examination in writing.
7) Subjects who are able to come to the study site on the designated examination date and undergo the examination.
8) Subjects who are deemed appropriate by the investigator to participate in the study.

Key exclusion criteria for the subjects as follows:

1) Subjects currently receiving drug treatment for any disease.
2) Subjects who have taken or applied drugs in the past month for the purpose of treatment of disease (excluding abrupt application of drugs for headache, menstrual cramps, common cold).
3) Subjects with a history or current history of mental illness, sleep disorders, hypertension, diabetes, dyslipidemia, or other serious illnesses.
4) Subjects with a history or current medical history of serious disorders of the liver, kidney, heart, lungs, blood, etc.
5) Subjects with co-morbidities or a serious history of gastrointestinal diseases.
6) Subjects who use artificial tears (eye drops) more than 6 times a day on a daily basis.
7) Subjects who have been diagnosed with presbyopia or are aware of presbyopia.
8) Subjects with ocular surface disease, entropion, or trichiasis.
9) Subjects with diagnosed dry eye.
10) Subjects who are using eye drops for the treatment of ocular diseases.
11) Subjects with refractive errors that have not been properly corrected.
12) Subjects who have undergone corneal surgery such as LASIK (laser in situ keratomileusis).
13) Subjects with severe astigmatism (> 2.0 D).
14) Subjects whose eye fatigue is caused by neurological or other regulatory dysfunctions.
15) Subjects with a body mass index (BMI) of 30.0 kg/m^2 or higher.
16) Subjects with drug or food allergies.
17) Subjects who have currently or within the past three months or will take functional foods, health foods, or supplements claiming to improve eye-related functions on a regular basis during the study period.
18) Subjects who have currently or within the past three months, or will take health foods containing rooster comb enzyme degradation products, hyaluronic acid (HA), collagen, proteoglycans, elastin, or their precursors on a regular basis during the study period.
19) Subjects whose daily alcohol consumption exceeds an average of 60 g/day of pure alcohol equivalent.
20) Subjects who have perennial allergy or have a risk of seasonal allergy such as pollen allergy during the study period.
21) Subjects who may change their lifestyle during the test period.
22) Subjects who work at night.
23) Subjects pregnant, lactating, or possibly pregnant.
24) Subjects who are currently participating in another human clinical trial or who have not yet completed 3 months of participation in another human clinical trial.
25) Subjects whose family members are engaged in the development, manufacture, or sale of health/functional foods and cosmetics.
26) Other subjects who are judged by the investigator and subinvestigators to be unsuitable for this study.

3. Selection of research participants, randomization and blind test

A mong candidates who the investigator and subinvestigators judged as appropriate, satisfying the inclusion criteria and not matching the exclusion criteria, thirty subjects were selected for the trial of the present study in order of the highest scores in QOL score for Questionnaire of Dry Eye related Quality of life Score (DEQS). Furthermore, for participants' selection, their backgrounds as well as other indexes were considered (***Fig. 1***).

Gender, age at the prior test, and QOL score in DEQS were stratified factors. After randomly allocating participants into two groups by Stratified Block Randomization, it was finally confirmed that there were no significant differences between the two allocated groups.

Two food products were employed for the trial: the test product was CRISTA®, which contained a rooster comb enzymatic degradation product in a high concentration, and the control food product was the product which did not contain a rooster comb enzymatic degradation product in a high concentration (Free of HA and collagen). The test sponsor made sure that products were unable to be distinguished by their packages, printing an identification symbol on products and sealing up them. They sent products with identification unseen to the tester, who confirmed that each product sent from the producer was unable to be recognized even if they smelled products or observed the appearance of packages. The tester then newly marked with another type of symbols for food usage management, on both test food and control food. This replacement enabled the blind clinical trial. Both products were handed over to a person responsible for management of the food products. The tester kept both the correspondence table sheet and the symbols for management. They were sealed and kept in a locked storage until the storage was unlocked and opened.

4. Test food product

Test food product was a capsule containing a high concentration of rooster comb enzymatic degradation product. Daily ingestion quantity was four 100 mg capsules. The method of ingestion as follows: Two capsules were taken by subjects twice a day with water or lukewarm water for four weeks. The main components were shown in ***Table 1***. Analysis of the laboratory center of additive free food cooperative marketing association confirmed one 100 mg capsule contains 2.375 mg of HA in total and 43.6 mg of collagen in total (Dosage quantity for one day: HA 9.5 mg, 174.4 mg).

Table1. Total composition and content of test food (250 mg/1 capsule).

Ingredient name	Test food	Placebo food
INJUV® (mg)	100	—
Vegetable oil (mg)	135	250
Other excipients (mg)	15	—

5. Study design

The present study employed a double-blind randomized controlled clinical trial with parallel-group comparison.

Primary endpoints were Dry Eye related Quality of life Score (DEQS), Visual Analogue Scale (VAS) regarding subjective symptoms, Tear break-up time and Schirmer's test. Secondary endpoints were visual acuity test, intra-ocular pressure, and Anti-aging QOL Common Questionnaire (AAQOL). To verify safety of the trial, examinations were performed such as ophthalmoscopy, slit-lamp microscopy, refractometry, blood pressure/pulse, consultation with a doctor, and assessments which were performed regarding absence/presence of adverse medicine reactions and adverse events.

Alcohol consumption and excessive exercise were not allowed for trial participants on the day before the test. Moreover, they were required to have sufficient sleep. On the day of the test, usage of artificial tears (eye drops) was not allowed until the completion of the test.

During the test period, usage of contact lenses was not allowed. The quantity of working length on a daily basis with visual display terminals (VDT) was maintained during the test period. The usage of eye drops as a measure for the prevention of dryness which was used until the test was not limited during the test period except for the day of the test day. Changing to another product was not allowed. In an unavoidable change, participants were required to record any changes in a participant journal. Eye drops containing HA were not allowed.

Trial participants were required to avoid irregular lifestyles such as insufficient sleep and excessive eating and drinking. In addition, they were supposed to maintain their habits in eating, doing exercise and sleeping quantitatively and qualitatively to be the same as before the commencement of the test.

Medicine, including external agents, quasi-pharmaceutical products, and herbal medicine were prohibited in principle. For unavoidable usage, they were required to report it to the consulting counter, recording the name of products used, the manufacturer, and the reason for the usage in participant journal. It was prohibited to start using or ingesting a new product of foods with functional claim, health food products, and supplements during the trial. When participants had regularly used health food products for the health maintenance before the participation in the trial, they were required to continue using them, not changing the method and usage quantity of products. In an unavoidable usage, they should record it in the participant journal stating the name of product used, its manufacturer, and the reason for the usage.

It was prohibited for participants of the present trial to newly participate in another human clinical trial within the timeframe of trial initiation to the completion of the test.

6. Examination Items

Questionnaire of Dry Eye related Quality of life Score (DEQS)

In the same method as the previous report [3], research participants answered DEQS questions for frequency of symptoms first; fifteen symptoms consisting of six "eye symptoms" and nine "influences to their daily life" (Column

A). After participants answered column A, the participants, who answered that they had any symptoms, recorded answers to questions (Column B) regarding whether or not they were concerned about them, and the severity of the problems. "QOL Score" was calculated with the formular: total score of column B / the number of valid responses × 25. We investigated severity in dry eye symptoms, impacts on daily life and psychological impacts.

Visual Analogue Scale questionnaire on subjective symptoms (VAS)

In the same method as the previous report [3], VAS investigations were conducted. Participants reported degrees of their conditions during past one week such as "eye dryness," "eye irritation," "eye pain," "blurred vision," "eye fatigue," "visual clarity," "stiff shoulders," "headache," and "sleep quality," employing the VAS scale of 100 mm line with the left side as the best condition (no symptoms) and the right side as the worst condition (the most severe symptoms they had experienced).

Anti-aging QOL Common Questionnaire (AAQOL)

Assessment in subjective symptoms employed AAQOL [6, 7]. Participants answered 33 questions regarding physical conditions and 21 questions regarding mental conditions, choosing one answer among five levels: 1) never, 2) rarely, 3) sometimes, 4) middle-level frequency, and 5) high-level frequency. In addition, they answered six questions regarding lifestyle habits, recoding numerical values.

The following tests of eye conditions were performed evaluating a dominant eye for assessments. All the test items were calculated with values of dominant eyes and non-dominant eyes, the mean values of left and right eyes, and the mean values of both eyes. All eye tests were performed in a medical institution and a board-certified ophthalmologist accessed the data.

Tear break-up Time Test (BUT)

In the same method of the previous report [3], participants were asked not to blink. The time of tear break-up was measured (measure time in seconds); the time taken for the first dry spot to appear on the cornea after a complete blink. The dry spot gradually expanded.

Schirmer' test

In the same method of the previous report [3], a prescribed strip of filter paper was placed inside the lower eyelid of participants. Their eyes produced tears and the strips absorbed them. After five minutes, the amount of moisture, length of wetness on the strip, was measured in millimeters.

Visal acuity test

Using a NIDEK space saving chart, SSC-370 Type D, unaided and best corrected visual acuity were measured on the right and the left eyes.

Tonometry test (measurement of intra-ocular pressure)

Using a Tomey MR-6000 multifunction refractometer, intra-ocular pressure of both the right and the left eyes were measured three times. When an outlier was recognized due to blinks, tonometry was measured once again. Adoped mean were the mean value of three tonometry measurement values excluding outliers.

In addition, the absence/presence of abnormal findings were examined by an ophthalmoscopy test (using a NEITZ rechargeable type brightscope, BSIII LED), a slit lamp microscopy test (using Takagi slit-lamp microscope, 700GL 0814040), and a refraction test (using Tomey MR-6000 multifunction refraction refractometer).

Statistical analysis

As fundamental statistics, mean value, standard deviation, standard error, maximum value, and minimum value were calculated. Examination result data were compiled to a summary sheet using Microsoft Office Excel 2016 (Microsoft Corp.). Values for analysis were measured values and alternation quantity compared with values prior to product ingestions. Statistical analysis software such as SAS 9.4 (SAS Institute Inc.), and SPSS Statistics 26 (IBM) were employed. Significance level of all tests was 5% with a two-sided test. Tendency level was 10%. Calculated data were expressed with mean value ± standard error to record in tables and graphs.

For comparison between two groups, the test food group and the placebo group, data from all tests were analyzed via t-test (unpaired). The unpaired t-test used P-Value as a homogeneity of variance. Scores obtained in DEQS and AAQOL were regarded as nonparametric. For group comparisons, Mann-Whitney U test was performed.

A paired t-test was performed for statistical data, comparing measured values at time of commencement (before ingestion) and all observation points (during observation period). Scores obtained in DEQS and AAQOL were regarded as nonparametric. For group comparisons, a Wilcoxon signed-rank test was performed.

Results

Classification and content of participants

The present study started with 30 subjects and was completed with 30 subjects, which included no excluded cases. Subjects mentioned below were chosen as the target of statistical analysis in the clinical case conference.

Analysis target for safety assessment/Intention to Treat (ITT) included 30 subjects: 15 subjects in the test products group, who ingested test food products at least once, and 15 subjects in the control product group.

Analysis target of effectiveness assessment/Per Protocol Set (PPS) included 29 subjects: excluding one subject from ITT analysis target (a subject of the test product group who met the exclusion criteria), 14 subjects of the test product group (male: seven, female: seven, age: 38.2 ± 3.2 years old) and 15 subjects of the control product group (male: eight,

female: seven, age: 38.4 ± 2.7 years old). The analysis flow of subjects is shown in ***Fig. 1*** and background information is shown in ***Table 2***. It is considered that the present experiment was not affected by six subjects with eye drops (three subjects of the test product group who used commercially available eye drops and three subjects of the control group who used commercially available eye drops), because the quantity of pharmaceutical ingredients were trace amounts in the eye drops.

Questionnaire of Dry Eye related Quality of life Score (DEQS)

Condition of DEQS results changes are shown in ***Table 3***. The following are items which suggested improvements in "Items regarding eye symptoms, which consisted of six items" among the DEQS for the test product group, comparing before ingestion and after two weeks: two items in column A, "dry eyes" ($p < 0.05$) and "eyes fatigue" ($p < 0.01$) and in column B, "dry eyes" ($p < 0.01$). At four weeks, for the test group, four items in column A: "dry eyes" ($p < 0.01$), "eyes fatigue" ($p < 0.01$), "heavy eyelids" ($p < 0.05$), and "eyes redness" ($p < 0.05$) and in column B: two items, "dry eyes" ($p < 0.05$) and "eyes fatigue" ($p < 0.05$). However, there were no significant differences between the two groups for either item.

Improved items in DEQS for the test food product group regarding "Impacts on daily life" (which consisted of nine items) were, comparing before ingestion and after two weeks, three items in column A: "worsened eye symptoms when watching TV or using a computer or cell phone" ($p < 0.05$), "lowered concentration due to eye symptoms" ($p < 0.01$), and "hindrance to work, housework, or study due to eye symptoms," and one item in column B: "lowered concentration due to eye symptoms" ($p < 0.05$). At four weeks, there were three improved items in column A: "worsened eye symptoms when watching TV or using a computer or cell phone" ($p < 0.05$), "lowered concentration due to eye symptoms" ($p < 0.01$), and "hindrance to work, housework, or study due to eye symptoms" ($p < 0.05$), and in column B, "lowered concentration due to eye symptoms" ($p < 0.05$). However, there were no recognized significant differences between the two groups in either item.

QOL scores, calculated with scores in column B, which were related to "degree", showed significant improvement in the test food product group: before ingestion 46.3 ± 3.5, after two weeks 29.8 ± 5.0, and after four weeks 26.4 ± 4.7 (both two weeks and four weeks: $p < 0.01$). In the control product group, scores also improved: before ingestion 51.3 ± 4.8, after two weeks 30.5 ± 4.2, and after four weeks 26.5 ± 4.4 (both two weeks and four weeks: $p < 0.01$). At two weeks and four weeks, no significant difference was recognized between the two groups.

Visual Analogue Scale questionnaire on subjective symptoms (VAS)

The changes of VAS are shown in ***Table 4***. The following is improved items in VAS (nine items): Comparing with one week before-ingestion, the four improved items were "dry eyes" ($p < 0.01$), "eyes fatigue" ($p < 0.01$), "stiff shoulder" ($p < 0.05$), and "headache" ($p < 0.05$). At two weeks, four improved items were "dry eyes" ($p < 0.05$), "eyes fatigue" ($p < 0.01$), "stiff shoulder" ($p < 0.01$), and "headache" ($p < 0.01$). At three weeks, five items were "dry eyes" ($p < 0.01$), "hazy and cloudy vision" ($p < 0.05$), "eyes fatigue" ($p < 0.01$), "stiff shoulder" ($p < 0.01$), and "headache" ($p < 0.05$). At four weeks, seven items were "dry eyes" ($p < 0.01$), "eye irritation" ($p < 0.05$), "hazy and cloudy vision" ($p < 0.05$), "eyes fatigue" ($p < 0.01$), "stiff shoulder" ($p < 0.01$), "headache" ($p < 0.01$) and "depth of sleep" ($p < 0.05$). No significant difference was recognized between the two groups.

Tear break-up time Test (BUT)

The changes in BUT are shown in ***Table 5***. Non-dominant eye tear break-up time was significantly improved in the test food product group from before ingestion (5.6 ± 0.4 seconds) to four weeks (7.3 ± 0.7 seconds) ($p < 0.05$). The test results for all eyes also significantly improved in the test product group from before ingestion (5.4 ± 0.3 seconds) to four weeks (6.8 ± 0.6 seconds) ($p < 0.05$). There was no significant difference in the control product group at both observation times. In the control group, no significant difference was recognized between non-dominant eye and both eyes. There was no significant difference between the two groups.

Schirmer' test

The changes in secretion volume of aqueous tears, are shown in ***Table 6***. The length of moisture on the filter paper for a dominant eye in Schirmer' test for the test food product group was 7.8 ± 1.3 mm before the ingestion. After four weeks, it was 15.1 ± 2.9 mm ($p < 0.05$). Similarly, in the test product group, non-dominant eyes test showed from 7.9 ± 1.2 mm before ingestion to 13.6 ± 2.8 mm after four weeks ($p < 0.05$). The test for mean value of the left and right eyes showed from 7.86 ± 1.07 mm before ingestion to 14.39 ± 2.78 mm after four weeks ($p < 0.05$). The test for all eyes showed from 7.9 ± 0.8 mm before ingestion to 14.4 ± 2.0 mm after four weeks ($p < 0.01$). Significant improvements were observed in all measurement items of lacrimal secretion volume. The test product group showed a significantly high value in comparison with the control product group regarding both-eyes measurement after four weeks ($p = 0.030$).

Table2. Profiles of subjects (PPS).

Group		Total	Male	Female
Test food	n	14	7	7
	Age	38.2 ± 3.2	44.3 ± 4.4	32.1 ± 3.8
	QOL Score (–)	46.3 ± 3.5	43.4 ± 3.9	49.1 ± 5.9
Placebo food	n	15	8	7
	Age	38.4 ± 2.7	40.5 ± 4.2	36.0 ± 3.3
	QOL Score (–)	51.3 ± 4.8	45.0 ± 6.0	58.4 ± 7.0

Data are shown as the mean ± SEM.

Table 3. Results of item (DEQS).

Item	Group	Before	p value (between-group comparison)	2 weeks	p value (vs. Before)	p value (between-group comparison)	4 weeks	p value (vs. Before)	
1) Foreign body sensation (Column A)	Test food	1.9 ± 0.3	0.404	1.3 ± 0.2	0.101	0.273	1.1 ± 0.3	0.087 †	0.819
	Placebo	2.1 ± 0.2		0.9 ± 0.2	0.002 **		1.0 ± 0.2	0.003 **	
1) Foreign body sensation (Column B)	Test food	2.3 ± 0.2	0.379	1.9 ± 0.2	0.236	0.348	1.9 ± 0.3	0.317	0.927
	Placebo	2.5 ± 0.3		1.6 ± 0.2	0.003 **		1.9 ± 0.2	0.014 *	
2) Dry sensation in eyes (Column A)	Test food	2.9 ± 0.1	0.450	1.9 ± 0.3	0.011 *	0.475	1.6 ± 0.3	0.004 **	0.874
	Placebo	2.9 ± 0.3		2.1 ± 0.2	0.032 *		1.7 ± 0.3	0.004 **	
2) Dry sensation in eyes (Column B)	Test food	2.9 ± 0.2	0.089 §	1.9 ± 0.2	0.005 **	0.389	1.7 ± 0.2	0.012 *	0.659
	Placebo	3.3 ± 0.2		2.2 ± 0.2	0.005 **		1.9 ± 0.2	0.003 **	
3) Painful or sore eyes (Column A)	Test food	1.4 ± 0.3	0.735	0.9 ± 0.3	0.107	0.816	0.9 ± 0.3	0.114	0.868
	Placebo	1.3 ± 0.3		0.9 ± 0.2	0.164		0.8 ± 0.3	0.203	
3) Painful or sore eyes (Column B)	Test food	2.2 ± 0.3	0.054 §	2.1 ± 0.3	1.000	0.713	2.0 ± 0.4	0.414	1.000
	Placebo	3.0 ± 0.3		2.0 ± 0.3	0.066 †		2.0 ± 0.2	0.046 *	
4) Ocular fatigue (Column A)	Test food	3.4 ± 0.2	0.884	2.2 ± 0.3	0.003 **	0.617	2.1 ± 0.3	0.004 **	0.892
	Placebo	3.4 ± 0.2		2.5 ± 0.3	0.004 **		2.1 ± 0.3	0.002 **	
4) Ocular fatigue (Column B)	Test food	2.8 ± 0.3	0.078 §	2.3 ± 0.2	0.124	0.825	2.0 ± 0.3	0.039 *	0.699
	Placebo	3.4 ± 0.2		2.3 ± 0.2	0.003 **		2.1 ± 0.3	0.003 **	
5) Heavy sensation in eyelids (Column A)	Test food	2.1 ± 0.3	0.875	1.4 ± 0.3	0.093 †	0.437	1.2 ± 0.3	0.016 *	0.342
	Placebo	2.1 ± 0.4		1.1 ± 0.3	0.012 *		0.9 ± 0.3	0.006 **	
5) Heavy sensation in eyelids (Column B)	Test food	2.3 ± 0.3	0.628	1.7 ± 0.3	0.086 †	0.278	1.8 ± 0.3	0.121	0.778
	Placebo	2.5 ± 0.2		2.1 ± 0.3	0.257		2.0 ± 0.4	0.103	
6) Redness in eyes (Column A)	Test food	1.5 ± 0.3	0.347	0.8 ± 0.3	0.067 †	0.352	0.6 ± 0.3	0.026 *	0.805
	Placebo	1.1 ± 0.2		0.4 ± 0.2	0.041 *		0.6 ± 0.2	0.071 †	
6) Redness in eyes (Column B)	Test food	1.7 ± 0.3	0.370	1.5 ± 0.2	0.458	0.453	1.6 ± 0.4	0.157	1.000
	Placebo	1.9 ± 0.3		1.3 ± 0.3	0.317		1.6 ± 0.3	0.564	
7) Difficulty opening eyes (Column A)	Test food	0.9 ± 0.2	0.179	0.6 ± 0.2	0.103	0.595	0.4 ± 0.2	0.083 †	0.631
	Placebo	1.5 ± 0.3		0.9 ± 0.3	0.011 *		0.7 ± 0.3	0.008 **	
7) Difficulty opening eyes (Column B)	Test food	2.3 ± 0.3	0.631	1.8 ± 0.3	0.083 †	0.389	1.8 ± 0.4	0.257	0.546
	Placebo	2.4 ± 0.3		2.3 ± 0.4	0.317		2.2 ± 0.4	0.103	
8) Blurred vision when watching something (Column A)	Test food	1.9 ± 0.3	0.926	1.5 ± 0.3	0.328	0.169	1.3 ± 0.3	0.146	0.801
	Placebo	1.9 ± 0.2		1.0 ± 0.2	0.010 *		1.2 ± 0.3	0.038 *	

8) Blurred vision when watching something (Column B)	Test food	2.5 ± 0.2	0.704	1.9 ± 0.2	0.096 †	0.239	2.1 ± 0.3	0.180	0.781
	Placebo	2.6 ± 0.2		2.3 ± 0.2	0.096 †		2.0 ± 0.3	0.075 †	
9) Sensitivity to bright light (Column A)	Test food	1.4 ± 0.3	0.457	0.9 ± 0.3	0.121	0.981	0.8 ± 0.2	0.146	0.362
	Placebo	1.7 ± 0.3		0.7 ± 0.3	0.011 *		0.5 ± 0.2	0.007 **	
9) Sensitivity to bright light (Column B)	Test food	2.3 ± 0.4	0.547	1.8 ± 0.4	0.180	0.637	2.0 ± 0.4	0.180	0.478
	Placebo	2.1 ± 0.2		1.6 ± 0.3	0.084 †		1.6 ± 0.4	0.194	
10) Problems with eyes when reading (Column A)	Test food	1.6 ± 0.3	0.716	0.9 ± 0.3	0.079 †	0.691	0.9 ± 0.3	0.098 †	0.981
	Placebo	1.7 ± 0.3		1.1 ± 0.3	0.040 *		0.9 ± 0.3	0.018 *	
10) Problems with eyes when reading (Column B)	Test food	2.3 ± 0.3	0.707	1.9 ± 0.3	0.578	0.494	1.7 ± 0.3	0.083 †	0.325
	Placebo	2.5 ± 0.3		2.1 ± 0.2	0.129		2.1 ± 0.3	0.198	
11) Problems with eyes when watching television or looking at a computer or cell phone (Column A)	Test food	2.5 ± 0.2	0.470	1.6 ± 0.3	0.015 *	0.704	1.6 ± 0.3	0.022 *	0.822
	Placebo	2.7 ± 0.3		1.8 ± 0.3	0.006 **		1.5 ± 0.3	0.004 **	
11) Problems with eyes when watching television or looking at a computer or cell phone (Column B)	Test food	2.4 ± 0.2	0.185	1.9 ± 0.2	0.167	0.145	2.1 ± 0.2	0.527	0.370
	Placebo	2.9 ± 0.3		2.4 ± 0.2	0.046 *		1.8 ± 0.2	0.017 *	
12) Feeling distracted because of eye symptoms (Column A)	Test food	2.4 ± 0.2	0.504	1.3 ± 0.3	0.002 **	0.909	1.0 ± 0.3	0.003 **	0.677
	Placebo	1.9 ± 0.3		1.3 ± 0.3	0.104		0.9 ± 0.2	0.024 *	
12) Feeling distracted because of eye symptoms (Column B)	Test food	2.7 ± 0.2	0.742	2.4 ± 0.2	0.034 *	0.438	2.1 ± 0.1	0.034 *	0.302
	Placebo	2.8 ± 0.2		2.2 ± 0.3	0.039 *		2.0 ± 0.4	0.020 *	
13) Eye symptoms affect work (Column A)	Test food	1.9 ± 0.3	0.598	1.0 ± 0.3	0.020 *	0.926	0.9 ± 0.3	0.014 *	0.153
	Placebo	1.6 ± 0.3		0.9 ± 0.2	0.026 *		0.5 ± 0.2	0.006 **	
13) Eye symptoms affect work (Column B)	Test food	2.4 ± 0.3	0.219	2.4 ± 0.3	0.103	0.038 #	1.9 ± 0.4	0.063 †	0.937
	Placebo	2.8 ± 0.2		1.7 ± 0.2	0.008 **		2.0 ± 0.5	0.257	
14) Not feeling like going out because of eye symptoms (Column A)	Test food	0.1 ± 0.1	1.000	0.1 ± 0.1	1.000	0.301	0.1 ± 0.1	0.317	0.136
	Placebo	0.1 ± 0.1		0.0 ± 0.0	0.317		0.0 ± 0.0	0.317	
14) Not feeling like going out because of eye symptoms (Column B)	Test food	2.0	0.317	2.0	1.000	N/A	1.0± 0.0	0.317	N/A
	Placebo	3.0			N/A			N/A	
15) Feeling depressed because of eye symptoms (Column A)	Test food	0.7 ± 0.3	0.129	1.0 ± 0.3	0.234	0.539	0.5 ± 0.3	0.450	0.419
	Placebo	1.3 ± 0.3		0.8 ± 0.3	0.021 *		0.7 ± 0.2	0.020 *	
15) Feeling depressed because of eye symptoms (Column B)	Test food	2.2 ± 0.4	0.791	1.8 ± 0.2	0.157	0.310	2.3 ± 0.5	0.706	0.418
	Placebo	2.3 ± 0.3		2.3 ± 0.4	0.713		1.9 ± 0.3	0.257	
QOL score	Test food	46.3 ± 3.5	0.678	29.8 ± 5.0	0.002 **	0.810	26.4 ± 4.7	0.004 **	0.948
	Placebo	51.3 ± 4.8		30.5 ± 4.2	0.001 ***		26.5 ± 4.4	0.001 ***	

Data are shown as the mean ± SEM

※Comparisons with "before" were made using Wilcoxon's test; †$p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

※Comparisons between groups at each time point were made by Mann-Whitney's U test; §$p < 0.10$, # $p < 0.05$.

Table 4. Visual analogue scale.

Item	Group	Before	p value (between-group comparison)	1 week	p value (vs. Before)	p value (between-group comparison)	2 week	p value (vs. Before)	p value (between-group comparison)	3 week	p value (vs. Before)	p value (between-group comparison)	4 week	p value (vs. Before)	p value (between-group comparison)
1. Eye dryness	Test food	65.7 ± 2.8	0.280	48.9 ± 3.8	0.001 **	0.357	48.1 ± 5.7	0.011 *	0.663	41.1 ± 5.1	0.001 **	0.336	43.5 ± 7.0	0.009 **	0.940
	Placebo	71.3 ± 4.1		54.7 ± 4.9	0.020 *		52.2 ± 7.1	0.025 *		49.9 ± 7.2	0.012 *		44.2 ± 6.1	0.000 ***	
2. Eye irritation	Test food	53.4 ± 5.3	0.698	48.4 ± 4.9	0.511	0.049 #	44.2 ± 5.7	0.178	0.207	43.4 ± 6.2	0.241	0.417	33.1 ± 7.6	0.045 *	0.419
	Placebo	50.1 ± 6.2		32.7 ± 5.8	0.003 **		34.5 ± 5.0	0.012 *		35.9 ± 6.6	0.045 *		25.5 ± 5.5	0.000 ***	
3. Eye pain	Test food	37.6 ± 6.7	0.661	33.7 ± 6.4	0.629	0.493	31.9 ± 7.2	0.527	0.532	29.8 ± 7.2	0.401	0.581	24.4 ± 7.0	0.154	0.885
	Placebo	42.8 ± 9.3		40.3 ± 7.0	0.719		38.2 ± 6.9	0.599		35.3 ± 6.8	0.447		23.1 ± 5.6	0.011 *	
4. Blurred vision	Test food	59.1 ± 6.0	0.591	52.1 ± 5.3	0.269	0.858	47.4 ± 6.4	0.095 †	0.503	42.9 ± 6.5	0.029 *	0.963	39.2 ± 7.5	0.029 *	0.473
	Placebo	64.0 ± 6.6		53.5 ± 5.6	0.088 †		52.8 ± 4.8	0.188		43.3 ± 5.9	0.022 *		31.9 ± 6.8	0.000 ***	
5. Eye fatigue	Test food	73.4 ± 3.5	0.083 §	57.0 ± 5.0	0.006 **	0.348	50.8 ± 6.0	0.003 **	0.256	45.9 ± 6.1	0.003 **	0.422	44.8 ± 7.5	0.005 **	0.824
	Placebo	84.1 ± 4.8		64.9 ± 6.4	0.002 **		60.4 ± 5.7	0.001 **		53.0 ± 6.2	0.001 ***		47.1 ± 7.3	0.000 ***	
6. Visual clarity	Test food	50.0 ± 7.3	0.229	49.6 ± 5.1	0.953	0.412	44.5 ± 5.5	0.371	0.731	41.3 ± 6.3	0.213	0.411	39.7 ± 7.4	0.153	0.279
	Placebo	60.8 ± 5.0		43.1 ± 6.0	0.006 **		41.5 ± 6.4	0.002 **		34.4 ± 5.4	0.002 **		28.7 ± 6.6	0.000 ***	
7. Stiff shoulders	Test food	73.1 ± 4.5	0.257	64.4 ± 5.0	0.042 *	0.099 §	59.0 ± 5.5	0.008 **	0.050 §	50.6 ± 6.4	0.003 **	0.109	53.2 ± 7.1	0.005 **	0.211
	Placebo	61.3 ± 8.9		47.9 ± 8.1	0.029 *		38.4 ± 8.3	0.010 *		32.8 ± 8.5	0.005 **		38.5 ± 8.9	0.019 *	
8. Headache	Test food	56.9 ± 7.2	0.604	37.9 ± 7.1	0.024 *	0.586	34.4 ± 7.3	0.003 **	0.887	31.1 ± 7.2	0.021 *	0.638	28.6 ± 8.4	0.009 **	0.687
	Placebo	51.1 ± 8.3		32.6 ± 6.5	0.002 **		32.9 ± 6.7	0.022 *		25.9 ± 8.0	0.003 **		23.9 ± 8.0	0.002 **	
9. Sleep depth	Test food	55.4 ± 7.2	0.677	55.5 ± 4.8	0.981	0.010 §	48.9 ± 4.9	0.323	0.085 §	37.9 ± 7.3	0.107	0.382	35.6 ± 6.7	0.041 *	0.276
	Placebo	51.1 ± 7.2		33.5 ± 6.2	0.045 *		33.3 ± 7.0	0.017 *		29.1 ± 6.6	0.024 *		24.3 ± 7.7	0.008 **	

Data are shown as the mean ± SEM.
※Comparisons with "before" were made with a paird t-test; †$p < 0.10$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
※Comparisons between groups at each time point were made with an unpaired t-test; §$p < 0.10$, #$p < 0.05$.

Table 5. BUT (Tear film break-up time)

Item	Unit	Group	Before	p value (between-group comparison)	4 week	p value (vs. Before)	p value (between-group comparison)
Dominant eye	second	Test food Placebo	5.1 ± 0.5 5.2 ± 0.8	0.951	6.3 ± 1.1 5.9 ± 0.8	0.319 0.552	0.752
Nondominant eye	second	Test food Placebo	5.6 ± 0.4 6.3 ± 1.0	0.524	7.3 ± 0.7 6.1 ± 0.9	0.039 * 0.889	0.351
Binocular mean	second	Test food Placebo	5.36 ± 0.44 5.73 ± 0.80	0.689	6.79 ± 0.87 6.00 ± 0.74	0.123 0.782	0.496
All eyes	second	Test food Placebo	5.4 ± 0.3 5.7 ± 0.6	0.594	6.8 ± 0.6 6.0 ± 0.6	0.038 * 0.710	0.378

Data are shown as the mean ± SEM.
※Comparisons with "before" were made with a paird t-test.*: $p < 0.05$
※Comparisons between groups at each time point were made with an unpaired t-test.
※all eyes Test food n = 28, Placebo n = 30

Table 6. Schirmer's test.

Item	Unit	Group	Before	p value (between-group comparison)	4 week	p value (vs. Before)	p value (between-group comparison)
Dominant eye	mm	Test food Placebo	7.8 ± 1.3 7.6 ± 0.7	0.898	15.1 ± 2.9 9.8 ± 2.0	0.019 * 0.175	0.132
Nondominant eye	mm	Test food Placebo	7.9 ± 1.2 7.1 ± 0.8	0.582	13.6 ± 2.8 9.0 ± 1.3	0.046 * 0.136	0.133
Binocular mean	mm	Test food Placebo	7.86 ± 1.07 7.37 ± 0.68	0.698	14.39 ± 2.78 9.40 ± 1.54	0.026 * 0.136	0.121
All eyes	mm	Test food Placebo	7.9 ± 0.8 7.4 ± 0.5	0.623	14.4 ± 2.0 9.4 ± 1.2	0.002 ** 0.042 *	**0.030** #

Data are shown as the mean ± SEM.
※Comparisons with "before" were made with a paird t-test; *$p > 0.05$, **$p < 0.01$.
※Comparisons between groups at each time point were made with an unpaired t-test; #$p < 0.05$.
※All eyes; Test food (n = 28), Placebo (n = 30).

Secondary endpoints

There were no significant differences recognized between the test product group and the control group in the visual acuity test and the tonometry test. Assessments in AAQOL at four weeks compared with before ingestion showed as follows: in the test product group, three items where conditions were significantly improved were “eye fatigue,” “stiff shoulders,” and “muscle pains and stiffness” (all items: $p < 0.05$). In the control group, five significantly improved items were “eye fatigue” ($p < 0.01$), “cloudy and hazy vision” ($p < 0.05$), “stiff shoulders” ($p < 0.01$), “bad conditions of the skin” ($p < 0.05$), and “stomachache” ($p < 0.05$). The control group showed significantly lower values in comparison with the test product group. For two items of quantity changes at four weeks: “bad conditions of the skin” and “being inclined to catch a cold” were recorded ($p < 0.05$).

Assessment in safety

No abnormal findings in safety assessments were reported for both the test product group and the control product group. The following four cases were observed: “itchy eyes,” “subconjunctival bleeding,” “sinus condition,” and “slightly throbbing stomach pain”. However, all of these symptoms were minor and there was no factors indicating a causal relationship with test products. From the above, the safety of the test food product ingestion during the four-week trial was confirmed.

Discussion

Overview of results

The present study, with the purpose of examinations on the effectiveness and the safety for eye moisture via the test food product ingestion, conducted a double-blind randomized controlled clinical trial with parallel-group comparison. Research participants were healthy males and females aged between 20 and 59 years old who experienced dry eyes or eye fatigue on a daily basis. The test food product was orally ingested for four weeks. Effectiveness on eye moisturization was examined regarding related indexes. Primary endpoints were DEQS, VAS, BUT, and Schirmer' test. Assessments of secondary endpoints were visual acuity test, tonometry test, and AAQOL.

Safety of the present study was validated, examining ophthalmoscopy, slit lamp microscopy test, refraction test, blood pressure/pulse, consultation with a doctor, and absence/presence of occurrence of adverse medical reactions and adverse events.

Data results of the trial showed significant increase in the secretion of tears (all eyes) for the test group, in comparison with the control group (significant difference between two groups, $p = 0.030$). For BUT (all eyes), no significant difference between the two groups was observed. However, the break-up time was significantly extended only in the test group. Regarding assessments in subjective symptoms, improvements in effects (placebo effects) were shown in the control group (DEQS, VAS, and AAQOL). There was no significant difference between groups. Schirmer' test and BUT, which were the primary endpoints for dry eye, were objective indexes. Therefore, these results suggest that the test food product had positive impacts on ocular dryness.

Causes of dry eye symptoms

Functions of tears are to prevent dryness on the ocular surface, to supply oxygen and nutrients to ocular tissues, and to prevent invasion and infection of foreign bodies and bacteria. Moreover, tears lubricate and smooth the surfaces of cornea, which enables a clear vision to be sent to the brain. Therefore, the decrease of tears and the deterioration of its functions could induce the decline of functional visual acuity (values of one-minute continuous measurement of visual acuity).

Precorneal tear film is composed of three layers, the mucin layer, the water layer and the lipid layer (from the deep part of the surface of corneal epithelium).

The mucin layer: Mucins are produced by conjunctival goblet cells. A destroyed or damaged mucin layer may cause a deficiency of tear function.

The water (Aqueous) layer: The lacrimal glands produce the middle layer, the main component of tear film. The water layer accounts for 95% of tear film and contains a variety of components such as protein and hyaluronic acid (HA).

The lipid layer: The meibomian glands produce the oil rich component. The lipid layer creates the smooth surface of tears, prevents tears from evaporation, and plays an important role in the protection from infection.[12)]

The tear film, composed of these three layers, obtains driving force to flow due to nictation or blinking, moisturizes the ocular surface with the flow in the one direction, and then, flow into the lacrimal punctum in upper and lower parts inside the lid margin and through upper and lower punctum, lacrimal sac, nasolacrimal duct, and nasal cavity. The following mechanism is recognized according to Dry Eye Diagnostic Guidelines[8)].

Instability of precorneal tear film, due to diverse risk factors in the upper stream, causes corneal epithelium disorder via dry stress. Consequently, the deterioration of wettability due to damage to mucin on the surface of the epithelium triggers a core mechanism that reduces the stability of precorneal tear film. As the result of this core mechanism, inflammations are initiated, which exacerbate the epithelium disorder.

Roles of hyaluronic acid (HA)

Hyaluronic acid (HA), which is widely distributed throughout the body, is the main component of synovial fluid produced from synovial membranes, and is a constituent of articular cartilage aggrecan. Hyaluronic acid (HA) has diverse important functions such as water retention[9,10)], lubrications of joints[11)], and intercellular adhesion[12,13)]. As a component of tears, HA serves as a function of preventing ocular dryness[14,15)].

The level of hyaluronic acid in the blood is clinically applied to indicators for articular diseases such as osteoarthritis (OA) ahd rheumatoid arthritis (RA), and as well as hepatic fibrosis.

Blood concentration level of HA differs substantially between individuals: Matsuura et al. 30.1 ± 16 ng/mL[16)], Nakazono et al. 42.2 ± 45.6 ng/mL[17)], and Yamada et al. 33.7 ± 24.2 ng/mL[18)], according to previous reports. Patients with Osteoarthritis (OA) and rheumatoid arthritis (RA) have higher levels of HA. Synovial fluid concentrations of HA in healthy adults are lower than that of blood concentrations (3.4 ng/mL)[19)]. Tears concentrations of HA are slightly higher than 18.9 ± 12.6 ng/mg protein of healthy adults (in the range of 3.2-45.0 ng/mg protein) were slightly higher than blood concentraitions[20)]. There is no difference for tears concentration in spite of wearing contact lenses. However, there would be a possible risk factor for the aged to wear contact lenses, because lacrimal fluid concentration of HA are lowered due to aging[21)] and aged eyes tend to become dry easily.

Eye drops which contain HA are frequently used as ophthalmic drugs (eye drop formulation) for dry eyes. However, its mechanisim of action is not well known. A previous study with animal experiences reported that eye drops with HA improved a function to repair the ectocornea after ectocornea desquamation[22)]. Matrix metalloproteinases is related to diverse pathophysiology of corneal disorders. Against this enzyme, HA has inhibitory effects[23-26)]. It is assumed that HA has anti-inflammatory effects on lacrimal glands and corneas. Surveys on treatment patterns for patients with dry eyes syndrome in ophthalmology clinics in Japan have indicated that ophthalmic solutions containing HA is regarded as a major agent[27)].

When glycative stress is added and advanced glycation endproducts (AGEs) increase in the blood and the skin tissues, via receptor for AGEs (RAGE) stimulation, the formation and the secretion of inflammatory cytokine increases, and MMP

expresses. Consequently, contents of collagen and HA decrease. Therefore, to improve these conditions, it is effective to orally ingest products with a functional component that promotes the degaradation of AGEs. This product decreases the amout of AGEs, inflammatory cytokine, and the expression of MMP, which results in increasing contents of collagen and HA in the skin[28]. With a similar mechanism, it is assumed that glycative stress induced by diabetes would worsen dry eyes symdrome.

Role of collagen

We have assumed that endogenous collagen in corneal epithelial cells plays a promotive role for wound healing of the corneal epithelial[29]. Patients with underlying medical conditions such as diabetes, glaucoma, and aqueous-deficiency dry eyes, have fragile corneal tissues. It is assumed that there is a possibility of delayed cell adhesion in the corneal wound.

In lacrimal gland extracellular matrix of patients with chronic dacryoadenitis, type I, III, and V collagen were positive, fibronectin was positive in acinar cells and fibrous tissues. Type IV collagen was positive only in basal membranes[30]. Via administration of transforming growth factor β (TGFβ), only TGFβ1 became positive in acinar cells and fibrous tissues. Therefore, a possibility was suggested that acinar-cell derived TGFβ1 was related to the formation and deposition of extracellular matrix for chronic dacryoadenitis.

As is a unique case, chronic Graft Versus Host Disease (cGVHD), which is a major complication of hematopoietic stem cell transplant, has dry eyes syndrome as an ocular condition. To treat this syndrome, other than the control of inflammatory reaction, up-regulations of type Iα1 and IIIα1 collagens and nuclear factor-kB (NF-kB) in lacrimal glands are important[31]. Furthermore, under pathological condition, MMP, which has effects of collagen degradation, is synthesized from corneal epithelial cells, corneal stromal cells, and inflammatory cells. The expression of MMP exacerbates tear function[32].

The destruction of diverse network such as allergic reaction and inflammation of upper palpebral conjunctiva could trigger corneal epithelium disorder and delayed cell adhesion in the corneal wound. It is assumed that cornea epithelium, which is a barrier against the external environment, is destroyed, and then, the expression of corneal fibroblasts is activated by inflammatory cytokine in tears and cellular infiltrate, inducing the acceleration of MMP activation and excessive degradation of collagen[33].

A therapy currently drawing attention is a method of thermosensitive atelocollagen injection using a puncture needle for plug[34,35]. This method could solve dry eye problems. Even though the amount of tear secreted from lacrimal glands is insufficient, tears are drained through opening of lacrimal punctums. The insufficient tear volume results in a dry ocular surface. This therapy, by setting a kind of plug, aims to maintain the sufficient amount of tears on ocular surface. Thermosensitive atelocollagen is liquid at a low temperature, and becomes a jelly-like substance at body temperature. By injecting this type of collagen with a puncture needle into the lacrimal duct connected from lacrimal canaliculus, the drainage of tears can be controlled.

Under normal circumstances, up-regulation of collagen formation in lacrimal grands is required[31]. Normal state of extracellular matrix structure such as collagen is desirable at the lacrimal duct which is located downstream of the lacrimal canaliculus.

Mechanism of action

The patent publications of No. 6435072 in Japan and of No. US-2019-0307788-A1 in the U. S. A., which referred to the patent obtained by Laimu Co., Ltd. regarding INJUV®, a food product containing a rooster comb enzymatic degradation product, mentioned that test results of the human fibroblasts, which INJUV® was added, suggested significant proliferative effect of fibroblasts and significant promotive effect of collagen formation, in comparison with the control group (non-addition of the test products. This was *in-vitro* findings. However, it was suggested that low molecular weight hyaluronic acid (HA) and collagen peptide, which were contained in INJUV®, after intestinal absorption, proliferated fibroblasts in extracellular matrix, which led to the promotion of HA and collagen formation.

The major component of the test food product used in the present study was the same low molecular weight hyaluronic acid (HA) and collagen peptide as INJUV®. CRISTA® is a product where INJUV® is condensed at a high concentration. This product is characterized for the extremely low molecular weight (molecular weight: 380–5,000) of HA. An open-label pilot trial was conducted and suggested that significant improvements were indicated in not only subjective symptoms but also items of tea film break-up tests as well as in tonometry and visual acuity tests. Absorbed low molecular weight HA in the body exerted some type of effects on fibroblasts (proliferation activities and actions of activating synthetic enzyme for HA and collagen). Mechanisms of action in HA functions have not yet been clarified. Further studies are required. We believe that the findings of improvements in subjective and objective symptoms via oral ingestion of HA is promising. The previous report suggested that via the ingestion of INJUV®, symptoms of arthralgia of knees and lumbago were improved[3]. Improvements in dry eye syndromes would be similarly explained as the processes of arthralgia and lumbago.

Currently obtained findings indicated how collagen peptide exerts beneficial effects. The ingestion of collagen hydrolysate induces the expression of prolyl-hydroxyproline (Pro-Hyp) and hydroxypropyl-glycine (Hyp-Gly) in human blood, and the proliferation of fibroblasts as well as the promotion of HA synthesis[36,37]. Consequently, the long-term ingestion of collagen hydrolysate on an everyday basis improves conditions of the skin and joints[38]. Pro-Hyp, as a proliferative factor, plays an important role in wound healing[39].

These findings suggested the following possibility: Collagen, a test product, was orally ingested, and absorbed. Collagen-derived dipeptides (Pro-Hyp, Hyp-Gly) are expressed in blood, and they affected the fibroblasts around the lacrimal glands. Furthermore, there was another suggested possibility of the contribution to the rise of HA concentration in blood and tears. For lacrimal tissues, HA, via the inhibition of MMP activation, acts as an anti-inflammatory, and HA had beneficial effects on functions of tears formation, as is expected. Collagen-derived dipeptide promoted the formation of collagen and HA in fibroblast cells. The

anti-inflammatory activities (MMP activation inhibition) of endogenous and exogenous collagen maintained and improved lacrimal environment. These could lead to the increase in tears formation. Furthermore, another possibility was suggested that the increase of HA in tears enhanced the moisturizing function of tears, which led to the prevention of decrease of tears due to evaporation.

Research limitation

The present study did not measure tears concentrations of HA and collagen-derived dipeptides, and was unable to obtain information whether or not a functional component, which was contained in the test food product, really transferred into the tears. Further examinations are required. There are various sizes of HA. It is recognized that high molecular weight type HA is effective in tear production [14]. There is no information regarding activities of low molecular weight HA. Further examination is eagerly required. As the number of cases is limited in the present study, we intend to conduct larger-scale tests to verify data results. The present study employed data obtained in all eyes test. However, data of both eyes of one subject are corelated with each other. Therefore, it was necessary to take it into consideration.

Conclusions

We conducted a double-blind randomized controlled clinical trial with parallel-group comparison for the ingestion of a test food product, which contains a rooster comb enzymatic degradation in a high concentration to evaluate the effectiveness on eye moisturization and safety. The test food products were orally ingested for four weeks. The Schirmer' test showed a significant increase in lacrimal secretion volume four weeks after the commencement of ingestion. Thus, the effectiveness of the test products on eye moisturization was verified. Furthermore, the test food products were confirmed to be safe throughout the continuous four-week ingestion. It is expected that this test product is effective for the prevention of ocular dryness and safe as an oral-administration functional food.

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Conflict of interest declaration

This trial was appropriately performed by third-party institutions, Anti Aging Bank Co., Ltd. (Tokyo) and TES Holdings Co., Ltd. (Tokyo). Authors of the present study included an employee of Laimu Co., Ltd. Other than that, there are no personal interests to declare.

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