Glycative Stress Research

Online edition : ISSN 2188-3610
Print edition : ISSN 2188-3602
Received : April 20, 2022
Accepted : May 20, 2022
Published online : June 30, 2022
doi:10.24659/gsr.9.2_97

Original article

Effects of rooster comb degradation product containing low-molecular hyaluronic acid (INJUV) in individuals with eye dryness; open-label trial with no control group.

Masahiko Ayaki [1], Sachio Wakayama [2], Yoshikazu Yonei [3]

1) Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan
2) Laimu Corporation. Yokohama, Kanagawa, Japan
3) Anti-Aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences, Doshisha University, Kyoto, Japan

Abstract

***Purpose*:** An open-level study with no control was conducted to confirm the changes in subjective and objective symptoms and safety of a food test product (INJUV) containing a rooster comb enzymatic degradation product that contains low molecular weight hyaluronic acid (HA) as a major component.

***Methods*:** From 25 healthy subjects who were aware of dry or eye fatigue, 12 subjects with severe symptoms (male: 6, female: 6, age: 37.8 ± 10.1 years) were selected as subjects. The subjects orally ingested INJUV for 4 weeks, and eye examinations and questionnaires were conducted before and after ingestion, and the results were compared.

***Results*:** Subjective symptoms that improved significantly were "eye dryness," "eye pain," "eye fatigue," and "eyelid heaviness." The nine items that improved significantly in the Anti-Aging QOL Common Questionnaire were "eye fatigue," "blurred vision," "eye pain," "stiff shoulders," "muscle pain/stiffness," "lethargy," "skin problems," "headache," and "dizziness." Tear layer breakdown time improved from 3.6 seconds to 5.5 seconds ($p < 0.05$), intraocular pressure (IOP) decreased from 13.0 mmHg to 11.7 mmHg ($p < 0.01$), and diastolic blood pressure decreased from 73.3 mmHg to 68.9 mmHg ($p < 0.05$). No adverse events were observed during the study period.

***Conclusion*:** Oral intake of INJUV was shown to be safe and improve ocular symptoms, quality of life and ocular surface, as well as lower IOP and diastolic blood pressure.

***KEY WORDS*:** low molecular weight hyaluronic acid, dry eye, Dry Eye-related Quality of Life Score (DEQS), tear layer breakup time, intraocular pressure, blood pressure

Introduction

The test ingredient INJUV (hereafter referred to as "test food") used in this study is a powdered substance made by lyophilization of rooster comb enzyme degradation products, the main component of which is ultra-low molecular weight hyaluronic acid (HA), produced by room temperature enzymatic degradation of whole rooster comb containing high molecular weight HA. In the past, an open pilot study on arthralgia and lumbago showed improvement in subjective symptoms of arthralgia and knee joint pain, and an increase in the range of motion of the knee joint [1]. Analysis of the Anti-Aging Quality of Life Common Questionnaire (AAQol) for changes in subjective symptoms showed significant improvement in scores for ocular symptoms "eye fatigue" and "blurred vision" in addition to "arthralgia" and "lumbago." These symptoms are associated with the ophthalmic disease "dry eye," which led us to conduct this study. Dry eye is usually treated with eye drops, and oral pilocarpine hydrochloride and supplements containing lactobacillus and lactoferrin are also used, but they are not established management. Here, an open study without a control group was conducted on "those who are aware of eye dryness and eye fatigue" for the purpose of examining the effect of the test food on dry eye symptoms.

Contact Address: Professor Yoshikazu Yonei, MD, PhD
Anti-Aging Medical Research Center,
Graduate School of Life and Medical Sciences, Doshisha University
1-3, Tatara Miyakodani, Kyotanabe, Kyoto, 610-0394 Japan
TEL & FAX: +81-774-65-6394 e-mail: yyonei@mail.doshisha.ac.jp
Co-authors; Ayaki M, ayaki@keio.jp; Wakayama S, s.wakayama@laimu.jp

Glycative Stress Research 2022; 9 (2): 97-105

Method

Subjects

The subjects were 25 healthy men and women between the ages of 20 and 60 who were aware of eye dryness and eye fatigue in their daily lives. In the preliminary survey, 12 subjects were selected for the study in order of their QOL score in the DEQS (Dry Eye Quality of Life Questionnaire score)[2], who met the selection criteria and did not violate the exclusion criteria. The main study was initiated with 12 subjects as described above, completed with all 12 subjects without any discontinuations, and all subjects were included in the analysis. The age of the 12 subjects (6 males and 6 females) in the test food (INJUV-containing food) group was 37.8 ± 10.1 years (males: 37.8 ± 8.9 years, females: 37.7 ± 12.0 years), and the DEQS QOL score at allocation was 52.4 ± 13.6 (males: 56.5 ± 15.7, females: 48.3 ± 10.9). Selection and exclusion criteria are presented next.

Selection criteria

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 (*e.g.,* 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 corrected visual acuity of 1.0 or better in both eyes and not wear contact lenses or be able to change to glasses 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.

Exclusion criteria

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 (*e.g.*, 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 currently, or within the past 3 months, who have 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 currently, and within the past 3 months, who have 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 may change their lifestyle during the examination period.
21) Subjects who work at night.
22) Subjects pregnant, lactating, or possibly pregnant.
23) 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.
24) Subjects whose family members are engaged in the development, manufacture, or sale of health/functional foods and cosmetics.
25) Other subjects who are judged by the investigator to be unsuitable for this study.

Test food

The test food was "INJUV capsules" provided Laimu Corporation (Yokohama. Kanagawa, Japan). INJUV is a powdered substance made by lyophilization of rooster comb enzyme degradation products. This test food contains HA with ultra-low molecular weight (molecular weight 380 ~ 5,000) and other collagen peptides as characteristic main ingredients. HA is distributed throughout the body, but in joints, it is produced by synovial membranes and is a major component of joint fluid and also a component of articular cartilage aggrecan[3]. HA plays important roles in water retention[4, 5], joint lubrication[6], intercellular adhesion[7], and immunomodulation[8]. HA-containing eye drops are often used for dry eyes[9].

In this study, four capsules containing 150 mg of INJUV were taken twice daily with water or lukewarm water for four weeks (600 mg of INJUV twice daily = 1,200 mg/day).

Study design

The study format was an uncontrolled open study.

In this study, the DEQS, VAS test for subjective symptoms, BUT (tear film breakup time), visual acuity test, and intraocular pressure (IOP) test were conducted before and 4 weeks after intake of the test food as indicators related to eye moisture. Schirmer test, visual acuity measurement, and IOP measurement were conducted. In addition, a survey was conducted on the effects of the intake of the test foods on QOL using AAQol.

The safety of the test food after 4 weeks of continuous intake was confirmed by fundus examination, slit-lamp microscopy, blood pressure/pulse rate, physician interview, occurrence of adverse events/side effects, and subject logbooks. In addition, background investigation, predominant eye determination, and refraction examination were conducted in the preliminary examination.

The study period was from September 2021 to November 2021.

Assessment Items

Subjective symptom

DEQS

In the DEQS, subjects first responded to a total of 15 items, six related to "Eye symptoms" and nine related to "Impact on daily life," with questions asking about the frequency of the symptoms (Column A). Consequently, for subjects who answered that the symptoms were at least a little bothersome, they were asked to indicate whether the symptoms bothered them and to what extent (Column B), and the "QOL score" was calculated by summing the Column B scores/number of valid responses*25. The severity of dry eye symptoms and their "Impact on daily life" and mental health were evaluated by comparing before and 4 weeks after consumption.

Experiential VAS questionnaire on subjective symptoms (Visual Analogue Scale).

In the VAS test, participants were asked to respond to the degree of "eye dryness," "eye irritation." "eye pain," "blurred vision," "eye fatigue", "visual clarity," "stiff shoulders," "headache," and "sleep depth" for the past week using the VAS on a 100-mm line. The left end was defined as the best condition (no symptoms) and the right end as the worst condition (greatest ever symptoms). The results were evaluated by comparing before and one week after, 2 weeks after, 3 weeks after, and 4 weeks after consumption.

AAQol.

The AAQol[10,11] was used to evaluate subjective symptoms. The AAQol was divided into "physical symptoms" and "mental symptoms." Then, the scores were evaluated on a 5-point scale from 1 to 5, with a before-and-after comparison between before and 4 weeks after consumption of the test foods.

Eye examination

The following eye examinations were performed to determine the dominant eye, and all items were tabulated as dominant eye values and nondominant eye values, and evaluated by comparing before and after 4 weeks of intake of the test food. All eye examinations were performed at a medical institution and the results were evaluated by an ophthalmologist.

BUT

Subjects were asked to hold back blinking, and the BUT, or the time it takes for the BUT (a phenomenon in which the area not covered by stained tears on the cornea appears and gradually expands), was measured.

Schirmer's test

A defined filter paper was placed between the lower eyelids of the subjects and the length of wetting in 5 minutes was determined (measurement/mm).

Visual acuity test

Uncorrected and corrected visual acuity were measured in the right and left eyes. The dominant eye was determined and analyzed. Results of visual acuity were converted to LogMAR (logarithm of minimum angular resolution) from the decimal acuity values obtained in the measurements, followed by statistical analysis. The conversion formula is LogMAR = −Log10 (decimal acuity value). This is because decimal acuity is the reciprocal of the minimum visual angle at which two points can be distinguished, and statistical processing is not possible with the number as it is. For example, a decimal acuity of 1.2 is converted to −0.08 in LogMAR, 1.0 to 0.00, and 0.8 to 0.10, so a smaller LogMAR acuity value means better acuity. Results are shown for both LogMAR and the decimal acuity values obtained by re-converting LogMAR to decimal acuity values.

Refraction test

Examination was performed in a dark or dim room (light-shielding curtains on windows).

IOP test

Three measurements were taken for both the right and left eyes using a pneumatic tonometer. If an outlier occurred due to blinking, eyelash, etc., the measurement was repeated, and the average IOP of the three measurements excluding the outlier was calculated.

Safety

Fundus examination, slit-lamp microscopy, blood pressure/pulse measurements, and physician interview/adverse drug reaction/adverse event assessment were conducted.

Statistical analysis

Statistical analysis was performed using SAS (SAS 9.4; SAS Institute Japan, Minato-ku, Tokyo) or SPSS (Statistics 26; IBM Japan, Chuo-ku, Tokyo) statistical analysis software, and a paired-t test was performed for comparison with before intake. Scores obtained from the DEQS and the AAQol and values of visual acuity tests were treated as nonparametric, and Wilcoxon signed rank tests were performed for comparison to preintake. The significance level for all tests was set at 5 % for two-tailed tests.

Ethical standard

This study was conducted under the approval of the Human Study Ethics Committee at the "Glycation Stress Research Society" (GSE #2021010) in Nakano, Tokyo, Japan. Clinical trial pre-registration was conducted for this study (UMIN #000045484).

Results

Evaluation of subjective symptoms

After 4 weeks of consumption of the test food, subjective symptoms improved as follows.

DEQS

In the DEQS "Eye symptoms" (6 items), all 6 items in Column A, "eye irritation," "eye dryness," "eye pain," "eye fatigue", "eyelid heaviness," and "eye redness" were significantly improved ($p < 0.05$) at 4 weeks after intake compared to before intake of the test food. Column B also showed significant improvement ($p < 0.05$) for four items: "eye dryness," "eye pain," "eyes fatigue," and "eyelid heaviness."

In the "Impact on daily life" (9 items), the results after 4 weeks of intake compared to the results before the intake of the test foods were significantly improved ($p < 0.05$), "eye symptoms worsen when watching TV or using a computer or cell phone," "concentration is impaired due to eye symptoms," and "eye symptoms interfere with work, housework, or study." Column B also showed significant improvement ($p < 0.05$) for the two items "eye symptoms get worse when watching TV or using a computer/mobile phone" and "concentration is impaired due to eye symptoms."

The QOL score, calculated using the Column B (degree) score, improved significantly from 52.4 ± 13.6 before consumption of the test food to 23.6 ± 10.6 four weeks after consumption ($p < 0.01$, ***Table 1***).

*VAS test (**Table 1**)*

In the VAS test (9 items), "eye dryness," "blurred vision," "eye fatigue," "visual clarity," and "stiff shoulders" improved significantly ($p < 0.05$) after 1 week compared to before intake of the test food. After 2 and 3 weeks, "sleep depth" was also significantly improved ($p < 0.05$), in addition to the 5 items that were significantly improved after 1 week. After 4 weeks, 8 items including "eye irritation" and "eye pain" were significantly improved ($p < 0.05$).

*AAQol (**Table 2**)*

The AAQol was used to evaluate physical symptoms 4 weeks after compared to before intake of the test food. The results showed significant improvement ($p < 0.05$) in "eye fatigue," "blurred vision," "eye pain," "stiff shoulders," "muscle pain/stiffness," "tiredness," "skin problems," "headache," and "dizziness" 4 weeks after compared to before intake.

*Eye examination (**Table 1**)*

BUT test

BUT is a test to determine how long it takes for the tears covering the surface of the eye to begin to dry out. When the patient stops blinking and looks at the front of the eye, the surface of the eye gradually dries out and the shape of the surface becomes uneven. This time is measured as BUT. Normal BUT is 10 seconds or more; if it is less than 5 seconds, the patient is diagnosed with probable dry eye. BUT (binocular mean) was less than 5 seconds in 9 of the 12 subjects. Compared to the pre-food intake, the dominant eye improved significantly from 4.1 ± 2.6 before to 5.6 ± 2.6 after 4 weeks ($p = 0.194$), the non-dominant eye improved from 3.2 ± 1.3 before to 5.5 ± 2.3 after 4 weeks ($p < 0.01$), and the binocular mean improved from 3.63 ± 1.72 before to 5.54 ± 2.36 after 4 weeks ($p < 0.05$).

Schirmer's test

The Schirmer's test measures the amount of tears produced. The normal level is 10 mm or more, and if it is less than 5 mm, the volume is judged to be low. The subjects' pre values (binocular mean) were in the normal range (>10 mm) in 5 of the 12 subjects, with the lowest value being 2.0 mm. No significant change in tear fluid volume was observed after 4 weeks of intake compared to the pre-value.

Visual acuity test

The binocular mean corrected visual acuity was 1.2 or better in 12 of the 12 subjects, with a minimum value of 1.2. Binocular means of noncorrected and corrected visual acuity were not significantly different after 4 weeks of intake compared to before. ***Table 1*** shows both decimal acuity and LogMAR acuity.

IOP test

The reference value for IOP testing is 10 ~ 21 mmHg, with diurnal variation. IOP tests showed that 11 out of 12 patients were within the reference range. IOP significantly decreased from a previous value of 13.0 ± 2.4 in the dominant eye to 11.7 ± 2.2 at 4 weeks ($p < 0.05$), from 13.0 ± 2.3 in the non-dominant eye to 11.7 ± 1.8 ($p < 0.05$), and from 13.0 ± 2.2 to the binocular mean of 11.7 ± 1.9 ($p < 0.01$).

Safety endpoints

No abnormal findings were observed in fundus examination or slit-lamp microscopy after 4 weeks of intake compared to pre-food intake. Diastolic blood pressure significantly decreased from 73.3 ± 10.7 before consumption to 68.9 ± 9.7 after 4 weeks ($p < 0.05$).

Table 1. Results

Item	Before	4 weeks	p value
Age (years)	37.8 ± 10.1		
Blood pressure			
Systolic (mmHg)	117.7 ± 13.0	113.4 ± 13.0	0.219
diastolic (mmHg)	73.3 ± 10.7	68.9 ± 9.7	**0.013***
Pulse (/min)	76.5 ± 11.3	81.1 ± 14.8	0.241
DEQS	52.4 ± 13.6	23.6 ± 10.6	**0.002***
Visual Analogue Scale			
Eye dryness	67.7 ± 18.9	34.7 ± 17.2	**0.001***
Eye irritation	46.5 ± 28.8	25.1 ± 19.4	**0.021***
Eye pain	51.9 ± 28.3	27.7 ± 20.0	**0.017***
Blurred vision	68.6 ± 25.1	32.0 ± 24.4	**0.002***
Eye fatigue	84.8 ± 10.1	44.2 ± 19.8	**<0.001***
Visual clarity	60.5 ± 22.6	28.8 ± 21.6	**0.007***
Stiff shoulders	75.3 ± 23.1	37.6 ± 21.3	**<0.001***
Headache	46.9 ± 40.5	22.9 ± 21.4	0.058
Sleep depth	65.3 ± 25.8	31.9 ± 18.6	**0.013***
Eye examination			
BUT (second)			
Dominant eye	4.1 ± 2.6	5.6 ± 2.6	0.194
Nondominant eye	3.2 ± 1.3	5.5 ± 2.3	**0.001***
Binocular mean	3.6 ± 1.7	5.5 ± 2.4	**0.023***
Schirmer's test (mm)			
Dominant eye	10.7 ± 9.1	12.5 ± 7.8	0.280
Nondominant eye	11.4 ± 10.5	11.3 ± 6.9	0.972
Binocular mean	11.0 ± 9.4	11.9 ± 7.0	0.632
Visual acuity (uncorrected)[A]			
Dominant eye	0.43 ± 0.30 (0.36 ± 0.53)	0.46 ± 0.27 (0.34 ± 0.57)	0.43 ± 0.30 (0.36 ± 0.53)
Nondominant eye	0.42 ± 0.31 (0.38 ± 0.51)	0.43 ± 0.30 (0.37 ± 0.52)	0.42 ± 0.31 (0.38 ± 0.51)
Binocular mean	0.54 ± 0.52 (0.26 ± 0.29)	0.35 ± 0.22 (0.46 ± 0.65)	0.54 ± 0.52 (0.26 ± 0.29)
Visual acuity (corrected)[A]			
Dominant eye	1.22 ± 0.94 (−0.09 ± 0.03)	1.29 ± 0.90 (−0.11 ± 0.04)	0.250
Nondominant eye	1.22 ± 0.94 (−0.09 ± 0.03)	1.27 ± 0.90 (−0.10 ± 0.04)	0.500
Binocular mean	1.28 ± 0.94 (−0.11 ± 0.03)	1.22 ± 0.93 (−0.09 ± 0.04)	0.375
Spherical refraction (D)			
Dominant eye	−1.52 ± 2.85	−1.56 ± 2.78	0.658
Nondominant eye	−1.40 ± 2.75	−1.52 ± 2.77	0.082
Binocular mean	−1.46 ± 2.79	−1.54 ± 2.76	0.207
Cylindrrical refraction (D)			
Dominant eye	0.56 ± 0.66	0.58 ± 0.55	0.845
Nondominant eye	0.60 ± 0.52	0.52 ± 0.36	0.474
Binocular mean	0.58 ± 0.57	0.55 ± 0.43	0.771
IOP (mmHg)			
Dominant eye	13.02 ± 2.35	11.74 ± 2.18	**0.014***
Nondominant eye	12.96 ± 2.30	11.66 ± 1.75	**0.015***
Binocular mean	12.99 ± 2.20	11.70 ± 1.86	**0.004***

Results are expressed as mean ± SD, n = 12, *p < 0.05, paired-t test or Wilcoxon test. [A] LogMAR in parenthesis; Decimal visual acuity was converted to LogMAR (logarithm of minimum angular resolution; DEQS, Dry Eye-related Quality of life Score; BUT, tear film breakup time; IOP, intraocular pressure; SD, standard deviation.

Table 2. AAQol scores.

Physical symptoms			
Item	Before	4 weeks	p value
Eye fatigue	4.3 ± 0.6	2.9 ± 0.5	**0.003***
Blurred vision	3.4 ± 1.2	2.2 ± 0.7	**0.006***
Eye pain	3.1 ± 1.2	1.8 ± 0.7	**0.006***
Stiff shoulders	4.1 ± 1.2	3.1 ± 0.8	**0.025***
Mascular pain/stiffness	3.4 ± 1.3	2.6 ± 1.2	**0.039***
Palpitations	1.5 ± 0.7	1.4 ± 0.7	0.564
Shortness of breath	1.6 ± 0.7	1.5 ± 0.7	0.564
Tendency to gain weight	2.0 ± 1.0	2.1 ± 1.2	0.739
Weight loss/; thin	1.6 ± 0.9	1.3 ± 0.5	0.48
Lethargy	3.2 ± 1.4	2.3 ± 0.9	**0.026***
No feeling of good health	2.5 ± 1.0	1.9 ± 0.8	0.143
Thirst	2.1 ± 0.8	1.8 ± 0.8	0.083
Skin problems	2.5 ± 0.7	1.9 ± 0.9	**0.020***
Anorexia	1.7 ± 0.7	1.3 ± 0.5	0.059
Early satiety	1.9 ± 1.1	1.4 ± 0.7	0.109
Epigastralgia	1.8 ± 1.0	1.3 ± 0.6	0.131
Liable to catch cold	1.4 ± 0.5	1.6 ± 0.9	0.706
Coughing and sputum	1.7 ± 1.0	1.58 ± 0.8	0.578
Diarrhea	1.7 ± 0.8	1.6 ± 0.8	0.739
Constipation	1.8 ± 1.0	1.6 ± 0.7	0.257
Gray hair	1.9 ± 1.2	1.5 ± 0.7	0.103
Hair loss	2.0 ± 1.2	1.8 ± 1.2	0.083
Headache	2.8 ± 0.8	2.1 ± 0.9	**0.014***
Dizziness	2.3 ± 1.0	1.3 ± 0.5	**0.016***
Tinnitus	1.8 ± 0.8	1.8 ± 0.7	1
Lumbago	2.8 ± 1.1	2.7 ± 0.9	0.763
Arthralgia	1.7 ± 0.9	1.8 ± 0.8	0.706
Edematous	2.2 ± 1.1	1.9 ± 0.7	0.257
Easily breaking into a sweat	2.6 ± 1.2	2.4 ± 0.9	0.581
Frequent urination	1.8 ± 1.0	1.8 ± 0.8	1
Hot flush	1.5 ± 0.7	1.5 ± 0.8	1
Cold skin	2.5 ± 1.4	2. ± 1.4	0.748

Mental symptoms			
Item	Before	4 weeks	p value
Irritability	2.6 ± 1.2	2.1 ± 1.2	0.059
Easily angered	2.3 ± 1.1	2.1 ± 2.1	0.18
Loss of motivation	2.3 ± 1.1	2.1 ± 1.0	0.578
No feeling of happiness	2.0 ± 1.0	1.9 ± 0.9	0.564
Nothing to look forward to in life	1.8 ± 0.9	1.7 ± 1.0	0.157
Daily life is not enjoyable	1.8 ± 1.1	1.6 ± 0.8	0.157
Loss of confidence	1.8 ± 0.9	1.8 ± 0.9	0.564
Reluctance to talk with others	2.0 ± 1.2	1.8 ± 1.0	0.18
Depressed	1.9 ± 1.2	1.8 ± 1.0	0.564
Feeling of usefulness	1.6 ± 0.7	1.7 ± 0.8	0.564
Sallow sleep	2.8 ± 1.1	2.3 ± 0.8	0.196
Difficulty in falling asleep	2.3 ± 1.1	2.1 ± 0.9	0.366
Pessimism	1.8 ± 0.9	1.8 ± 1.1	1
Lapse of memory	2.3 ± 0.8	1.8 ± 0.8	0.16
Inability to concentrate	2.3 ± 1.1	2.1 ± 1.0	0.18
Inability to solve problems	1.8 ± 0.9	2.1 ± 1.0	0.408
Inability to make judgments readily	2.0 ± 1.0	2.0 ± 1.0	1
Inability to sleep because of worries	1.9 ± 0.9	1.8 ± 1.0	0.564
A sense of tension	2.1 ± 1.0	2.0 ± 1.0	0.706
Feeling of anxiety for no special reason	1.7 ± 0.7	1.8 ± 1.0	0.414
Vague feeling of fear	1.3 ± 0.5	1.5 ± 0.8	0.527

Results are expressed as mean ± SD, n = 12, *p < 0.05, Wilcoxon test. AAQol, Anti-Aging QOL Common Questionaries; SD, standard deviation.

Discussion

Result summary

Characteristics of the 12 men and women who experienced ocular dryness included low BUT (5 mm), normal tear fluid volume, generally normal corrected visual acuity, and IOP in the normal reference range. All patients met the new 2016 diagnostic criteria of "dry eye syndrome." BUT less than 5 seconds and subjective symptoms (ocular discomfort or abnormal visual function). After a 4-week test food feeding, the patients showed improvement in dry eye-related symptoms, prolonged BUT (binocular mean +34.5%), and decreased IOP (binocular mean −9.9%). No adverse events were observed.

Lacrimal function and dynamics of tear fluid

Tears protect the cells on the surface of the eye from drying out, supply nutrients and oxygen to the tissues, and prevent the entry of foreign substances and bacteria from the outside world. They also provide a smooth curve to the surface of the cornea and deliver clear images to the brain. Therefore, a decrease in tear fluid or a declined tear fluid function may result in a decrease in functional visual acuity (practical acuity value expressed as a continuous measurement over a period of one minute).

Lacrimal fluid consists of three layers from the depth (surface of the corneal epithelium): mucin layer, water layer, and oil layer.

Mucin layer: mucus secreted by the goblet cells of the conjunctiva. Lack of the mucin layer causes a loss of tear fluid from corneoconjunctival epithelium.

Water (Aqueous) Layer: The main component of tears secreted by the lacrimal gland. The aqueous layer accounts for 95% of tears and contains various components such as proteins and HA. It plays an important role in the nutritional supply to the cornea, prevention of infection, wound healing, and other important functions of tears.

Oil layer: Secreted by the meibomian glands and rich in oil components. It covers the surface of the tear fluid and serves to prevent evaporation of tears and to protect against infection. The lipids present in the oil layer are chain-like molecules called meibum, the main components of which are cholesterol esters and wax esters[12].

The three layers of lacrimal fluid are driven by the blinking eye and, after moistening the ocular surface in a constant direction, flow into the upper and lower lacrimal ducts on the inner side of the eyelid margin and then through the upper and lower lacrimal ducts, lacrimal sac, nasolacrimal duct, and nasal cavity.

According to dry eye practice guidelines[13], the following mechanisms are postulated. When the stability of the tear fluid layer is reduced by various upstream risk factors, epithelial damage to the corneal conjunctiva occurs due to drying stress. Consequently, mucins on the epithelial surface are impaired and water wettability is reduced. A vicious cycle (core mechanism) occurs in which the stability of the tear fluid layer is further reduced[14-18]. This vicious cycle further results in inflammation and contributes to epithelial damage.

Role of hyaluronic acid (HA)

The mechanism of action of HA, the main component of HA-containing topical products that are frequently used with eye drops for the treatment of dry eye, still remains unclear. In animal studies, HA-containing topical artificial tear solutions have been reported to improve corneal re-epithelialization after corneal epithelial peeling[19]. Biochemically, it has been speculated that phospholipid-HA interactions may be associated with vitreous liquefaction associated with aging, treatment of dry eye syndrome, skin care products, and synovial joint lubrication[20]. The affinity of HA with other substances is strongest with phosphatidylcholine sphingomyelin and phosphatidic acid, followed by monoglycerides and palmitoyl palmitate, and weakest with cholesterol[21].

In humans, its efficacy in increasing tear film thickness (TFT) has been reported in a randomized, double-masked, placebo-controlled study[22]. However, there is some argument that artificial tears containing HA only transiently alleviate dry eye symptoms, and combination agents with galactoxyloglucan have also been proposed[23].

In recent years, modern life has become very convenient with the spread of personal computers and air conditioning control, whereas, the environment surrounding the eyes has not improved. According to the Osaka Study reported in 2013, dry eye examinations and questionnaires were conducted on 561 company employees, and 79.5% of women and 60.2% of men were diagnosed with dry eye, which was more pronounced in the group using VDT for 8 hours or more per day[24]. Even under the pandemic of Covid-19 from 2020, dry eye is increasing[25]. Under these circumstances, an effective and safe treatment for dry eye that addresses the pathogenesis of dry eye is desperately needed.

Considering dry eye as a lifestyle-related disease, lifestyle modification is a prerequisite for prevention and treatment. Lifestyle guidance through dietary modification, increased physical activity, and encouragement of positive thinking has been shown to improve subjective symptoms associated with dry eye[26].

The test product used in this study, a food product containing HA, is characterized by its main ingredient, HA, being ultra-low molecular weight (molecular weight 380 ~ 5,000). Despite the fact that this was an open pilot study, not only subjective symptoms but also BUT and IOP were significantly improved. This effect may be due to the fact that the low molecular weight HA contained in the test product was absorbed into the body and had some effect. Although the mechanism of action of HA is currently unknown and requires further investigation, the fact that both subjective and subjective symptoms improved after oral intake is groundbreaking. In a previous report, symptoms of the knees and hips were improved by INJUV administration[1], and it is assumed that the effects on the ocular area were produced by a similar pathway.

Hypothesis raised for IOP and diastolic blood pressure reduction is as follows. Dry eye is closely related to mental stress[27] and the autonomic nervous system[28], and the effect of HA-containing foods on the ocular surface first improves BUT, which in turn improves optical function of the ocular surface and improves vision. It is possible that these

improvements in dry eye and vision may have reduced stress, resulting in a decrease in IOP[29,30] and diastolic blood pressure. IOP, like blood pressure, is supposed to fluctuate seasonally via catecholamines[31], and a reduction in IOP due to improvement of dry eye or other effects may be expected as a potential effect of INJUV.

HA is also detected in tear fluid. The average HA concentration has been reported to be 18.9 ± 12.6 ng/mg protein (range 3.2 ~ 45.0 ng/mg protein) in healthy subjects[32]. There are no differences between men and women or between contact lens wearers and non-wearers[33]. It is increased in patients with Basedow's disease (hyperthyroidism), reaching 35.1 ± 34.7 ng/mg protein[32]. Results of an analysis of tear fluid from patients with unilateral acute adenoviral conjunctivitis (UAAC), normal donors (controls), and allergic conjunctivitis showed that HA tear fluid volume was significantly increased in patients with UAAC compared to controls and allergic conjunctivitis patients[34]. These findings suggest that there may be a mechanism by which HA in the tear fluid is increased by some stimulus to maintain ocular homeostasis.

Research limitations.

Since this is an uncontrolled, open-label study, the level of evidence for the efficacy of this study with respect to dry eye is not high. There are many unknowns regarding the disposition of the main component of the test food, ultra-low molecular weight HA, in the body. Whether or not the HA concentration in the tear fluid is increased by the ingestion of the test food is a subject for future verification. The reason for the significant decrease in IOP and diastolic blood pressure was only hypothesized and should be further confirmed.

Safety

No abnormal findings were observed in fundus examination or slit-lamp microscopy after 4 weeks of intake compared to pre-food intake. Diastolic blood pressure significantly decreased from 73.3 ± 10.7 before to 68.9 ± 9.7 after 4 weeks of consumption compared to before consumption ($p < 0.05$). This variation was within the reference range. No significant changes were observed in systolic blood pressure or pulse rate. No other adverse event investigations were found to be causally related to the test food.

Conclusions

To confirm the changes in subjective and objective symptoms and safety of a food test product (INJUV) containing low molecular weight HA-based rooster comb enzymatic degradation products, an uncontrolled, open-level study was conducted in 12 healthy subjects (6 males, 6 females, age: 37.8 ± 10.1 years) who were aware of eye dryness and eye fatigue. As a result, subjective symptoms such as “eye dryness,” “eye pain,” “eye fatigue,” and “eyelid heaviness” were significantly improved. In the AAQol, 9 items significantly improved: “eye fatigue,” “blurred vision,” “eye pain,” “stiff shoulders,” “muscle pain/stiffness,” “lethargy,” “skin problems,” “headache,” and “dizziness.” Ocular examination showed an improvement in BUT, a decrease in IOP, and a decrease in diastolic blood pressure. No adverse events were observed during the study period, confirming safety. Since this is an open, uncontrolled study, results may include a placebo effect. Further detailed investigation of the efficacy evaluation and mechanism of action of the test product is needed in a randomized controlled trial (RCT).

Conflict of interest declaration

In carrying out this study, we received support from Laimu Co.

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