BPB Reports

Regular Article

Oral Administration of Heat-Killed *Fructobacillus Fructosus* FMO-85 Alleviates the Reduction in Tear Fluid in a Stress-Induced Dry Eye Mouse Model

Emi Sugisawa,[a] Wataru Otsu,[a] Tomohiro Yako,[b] Taketoshi Hata,[c] Tomomi Morimoto,[c] Hiroe Maruyama,[c] Hiroyuki Kono,[c] Shinsuke Nakamura,[b] Hideshi Tsusaki,[a] and Masamitsu Shimazawa[a,b,*]

[a]*Department of Biomedical Research Laboratory, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu, 501-1196, Japan;* [b]*Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu, 501-1196, Japan;* [c]*Nagaragawa Research Center, API Co., Ltd., 692-3 Nagara, Gifu, 502-0071, Japan*

Received December 30, 2024; Accepted March 28, 2025

Dry eye disease is an ocular disease in which the stability of tear fluid decreases, causing ocular discomfort and abnormal visual function as well as damage to the ocular surface. It has been reported that specific types of food ingredients can promote lachrymal secretion, which is expected to prevent and improve dry eye. Here, we evaluated the effects of a heat-killed form of *Fructobacillus fructosus* FMO-85, a species of fructophilic lactic acid bacteria (FLAB) that is derived from the digestive tract of honeybees, on lacrimal fluid secretion using a stress-induced dry eye mouse model. Male C57BL/6J mice were fed a 3% FLAB-mixed diet 3 weeks before stress loading. We observed that the tear fluid volume was decreased after stress loading, which was significantly improved by FLAB treatment after 7 and 11 days. Mechanistically, the mRNA levels of brain-derived neurotrophic factor (*Bdnf*), one of the important growth factors involved in lacrimal fluid secretion, isoform-2 and -6 were increased in the hippocampus of FLAB-treated mice. Furthermore, the plasma levels of an anti-inflammatory cytokine, interleukin-10 (IL-10), increased in the FLAB-treated mice. These results suggested that the ingredients contained in dried FLAB increase tear fluid volume by affecting the lacrimal secretion mechanism and the production of inhibitory cytokines. In conclusion, the decrease in tear fluid volume after stress loading was suppressed by FLAB intake. These results indicated that FLAB supplementation may be a useful strategy for the prevention and treatment of dry eye.

Key words fructophilic lactic acid bacteria, *Fructobacillus fructosus*, tear film, brain-derived neurotrophic factor, interleukin-10, corneal epithelium

INTRODUCTION

Dry eye is a disorder caused by reduced tear fluid retention due to decreased tear fluid secretion and leads to corneal cell damage. Prolonged exposure by facing visual display terminals (VDTs) is a risk factor for dry eyes, eye fatigue, and eye inflammation.[1–3] Therefore, many people are at potential risk for these ocular conditions because VDTs are indispensable in modern life and work. Currently, drugs approved for the treatment of dry eye target increased mucin secretion and corneal epithelial damage. There is a need for daily preventative measures potentially derived from foods with functional abilities to prevent stress-induced damage to the eyes.

Growing evidence suggests the interconnection between the gut microbiota and the central nervous system, including the eyes.[4] The gut microbiome is implicated in several ocular diseases, such as diabetic retinopathies,[5] glaucoma,[6] and age-related macular degeneration.[7] Lactic acid bacteria (LAB) are Gram-positive, non-sporulating, facultative anaerobic microorganisms that are known to promote gut functions of the host, including humans.[8] Interestingly, oral administration of *Lactobacillus fermentum*, which belongs to the genus of *Lactobacillus*, relieved benzalkonium chloride solution-induced dry eye,[9] suggesting that specific types of LABs could be beneficial for eye health, including ocular surface disorders.

The recently discovered fructophilic lactic acid bacteria (FLAB) have been characterized as a special group of lactic acid bacteria that prefer fructose instead of glucose as a carbon source.[10] Fructobacillus species, including *Fructobacillus fructosus*, are new species of Gram-positive lactic acid bacteria belonging to the family *Lactobacillaceae*, which grow in fructose-rich environments such as flowers, fruits, and

*To whom correspondence should be addressed. e-mail: shimazawa@gifu-pu.ac.jp

fruit ferments.[11] FLAB is associated with the gastrointestinal tracts of honeybees.[12] To adapt to the environments in the insect guts, the genomes of FLAB have acquired a deletion of the bifunctional alcohol/acetaldehyde dehydrogenase gene (*adhE*).[13] Recently, using an *in vitro* system, it has been shown that FLAB exhibits functional properties as a biocontrol agent, which regulates the growth of undesired microbes.[14] However, it remains unclear whether FLAB intake is beneficial for mammals, including humans.

Here, we focused our studies on the FLAB strain *F. fructosus* FMO-85, which is derived from the digestive tract of honeybees. We evaluated the effects of *F. fructosus* FMO-85 on lacrimal fluid secretion using a constrained blast dry eye mouse model. In addition, we examined the mRNA levels of brain-derived neurotrophic factor (*Bdnf)* isoforms in the hippocampus of FLAB-treated mice. To assess the impact of FLAB on the general immunity of the whole body, we analyzed the amount of interleukin-10 (IL-10) in the plasma of these mice.

MATERIALS AND METHODS

Bacterial Strain The FLAB strain *F. fructosus* FMO-85 was supplied by API Co. Ltd. (Gifu, Japan) as a sterilized heat-killed dried powder.

Animal Treatments C57BL/6J mice (male, five-week-old) were obtained from Charles River Japan, Inc. (Kanagawa, Japan). The schedule of the animal experiment is shown in Fig. 1A. Mice were fed either the American Institute of Nutrition (AIN)-93M standard purified diet (Oriental Yeast Co., Tokyo, Japan) or the AIN-93M diet mixed with 3% (w/w) of the sterilized and dried powder of FLAB from 3 weeks prior to air stress until the last day of the study (38 days after the start of feeding). A stress-induced dry eye model was established as reported previously with a minor modification.[15] In short, mice that had reached the age of 8 weeks were restrained in a treated polypropylene centrifuge tube that allowed breathing/defecation for 4 h per day, and air was applied to the face of each mouse during the restraint. This procedure was repeated for 4 days to induce dry eye symptoms. The mice were housed under a 12-h light-dark cycle until harvest. The experimental procedures were conducted according to the statement of the Association for Research in Vision and Ophthalmology (ARVO) for the Use of Animals in Ophthalmic and Vision Research. All experiments were approved and monitored by the Institutional Animal Care and Use Committee of Gifu Pharmaceutical University.

Tear Secretion Volume Mouse tear volume was measured before stress loading (days 0, 7, 14, and 20 after the start of feeding), at each day of stress loading (every day from days 21 to 24), and after the end of stress loading (days 25, 27, 29, 31, 34, and 38). The tear volume was calculated by inserting cotton phenol red threads (FCI Ophthalmic, Pembroke, MA, USA) into the external eye angle for 15 seconds and dividing the discolored length (mm) by the weight of the mouse (g).

Histological Analysis Histological analysis was carried out as reported previously.[16] Briefly, mice were euthanized with pentobarbital (150 mg/kg), and the mouse eyes were enucleated. The eyeballs were fixed in 0.1 M phosphate buffer (PB) containing 4% paraformaldehyde (pH 7.4) for 48 h at 4°C. Paraffin-embedded sections (thickness: 5 μm) were stained with hematoxylin and eosin. All the images were obtained using a BZ-X710 microscope (Keyence, Osaka, Japan). The corneal epithelium thickness was measured using photographs obtained with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Isolation of RNA and Real-Time PCR Total RNA was extracted from the mouse hippocampus using NucleoSpin RNA (Takara Bio, Shiga, Japan), and a real-time PCR experiment was performed according to the manufacturer's instructions. The cDNA library was created using a PrimeScript RT Reagent kit (Takara Bio, Shiga, Japan) and then was subjected to quantitative (q)PCR. The qPCR was performed on a Thermal Cycler Dice Real Time System III (Takara Bio, Shiga, Japan) using TB Green Premix Ex Taq II (Takara Bio, Shiga, Japan) with the primer pairs shown in Table 1. The CT values were normalized to *Gapdh* mRNA levels.

Enzyme-Linked Immunosorbent Assay (ELISA) Blood was collected from the mice under anesthesia. Plasma was obtained from the blood by centrifugation at 1,700 × *g* for 10 min. The level of IL-10 in the plasma was measured using the Mouse IL-10 Quantikine ELISA kit (M1000B-1; R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The absorbance was measured using a Varioskan LUX microplate reader (Thermo Fisher Scientific, Waltham, MA) at 450 nm with the correction wavelength set at 540 nm.

Statistics Test results are presented as the mean ± standard error of the mean (SEM). The SPSS Statistics software (IBM Armonk, NY, USA) was used for statistical comparisons. Statistical comparisons were performed using the Tukey test Student's *t*-test, or Games-Howell test. A p-value < 0.05 was considered statistically significant.

RESULTS

The Effects of a FLAB Strain on Tear Fluid Volume in a Stress-Induced Dry Eye Model The FLAB used in this study was the *F. fructosus* FMO-85 strain, which was sterilized in a heat-killed dry powder form. Five-week-old male

Table 1. List of Primer Sequences Used in This Study

Target Gene	Primer sequence (5'-3')	
	Forward	Reverse
Gapdh	5'-AGGAGCGAGACCCCACTAAC-3'	5'-GATGACCCTTTTGGCTCCAC-3'
Bdnf isoform 1	5'-CACATTACCTTCCTGCATCTGTTG-3'	5'-ACCATAGTAAGGAAAAGGATGGTCAT-3'
Bdnf isoform 2	5'-TTGGGAAATGCAAGTGTTTATCA-3'	5'-CGAAGTATGAAATAACCATAGTAAGGAAAA-3'
Bdnf isoform 4	5'-CTGCCTTGATGTTTACTTTGACAAG-3'	5'-ACCATAGTAAGGAAAAGGATGGTCAT-3'
Bdnf isoform 6	5'-CAGAAGCGTGACAACAATGTGA-3'	5'-ACCATAGTAAGGAAAAGGATGGTCAT-3'
Total Bdnf	5'-ACTATGGTTATTTCATACTTCGGTT-3'	5'-CCATTCACGCTCTCCAGA-3'

The primer pairs for each isoform of mouse *Bdnf* were designed as reported previously.[15]

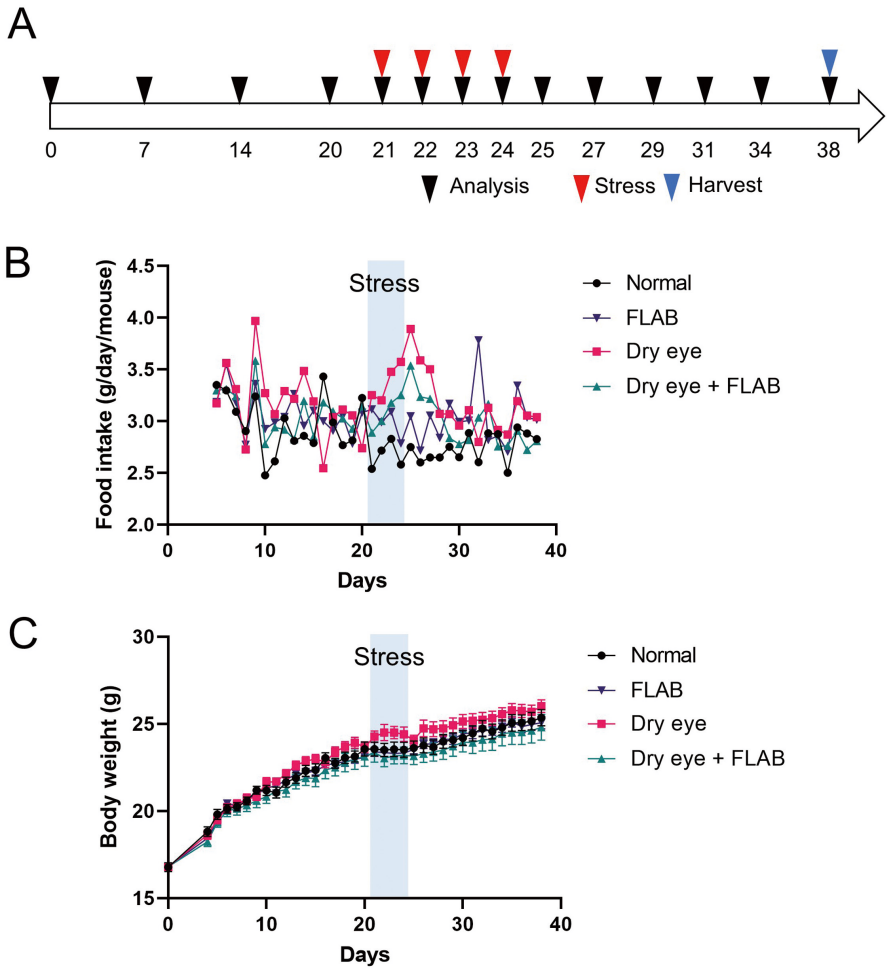

Fig. 1. Murine Food Intake and Body Weight Changes during the Study of the Effects of FLAB on a Stress-Induced Dry Eye Model

(A) The protocol for investigating the effects of *F. fructosus* FMO-85 on the stress-induced dry eye model. Black, red, and blue arrowheads indicate the time points of tear secretion volume measurement (Analysis), stress loading (Stress), and tissue harvest (Harvest), respectively. (B,C) The graph shows the average food intake (g/day/mouse; B) and body weight (g; C) in each group [Normal, AIN-93M diet without stress loading (black); FLAB, AIN-93M diet containing 3% FLAB without stress loading (purple); Dry eye, AIN-93M diet with stress loading (red); and Dry eye + FLAB, AIN-93M diet containing 3% FLAB with stress loading (green)]. The data comprise the means ± SEMs (n = 6-10).

C57BL/6J mice were used. The mice were provided with free access to the AIN-93M diet or the AIN-93M diet mixed with 3% dried FLAB from 3 weeks prior to air stress until the day of tissue harvest and proceeding to the last day of the study (38 days after the start of feeding) (Fig. 1A). Eight-week-old mice were restrained in a treated polypropylene centrifuge tube for 4 h per day, and a blast of air was applied to the mouse face during restraint. This procedure was repeated for 4 days to induce damage. The FLAB-supplemented diet continued for 2 weeks after stress loading. The food intake was approximately 3 grams per day for each group, and there were no significant differences in the food intake among groups (Fig. 1B). We also found no significant differences in growth between the groups with or without FLAB supplementation (Fig. 1C).

As shown by the black arrows in Fig. 1A, the lacrimal fluid volumes were measured using cotton phenol red thread test (Fig. 2A) before blast stress (days 0, 7, 14, and 20 after the start of feeding), at each day of blast stress (every day from days 21 to 24 after the start of feeding), and after the end of the blast treatment (days 25, 27, 29, 31, 34, and 38 elapsed days from the start of feeding). The lacrimal fluid was significantly decreased after stress loading. Moderate improvement of the lacrimal fluid volume was observed between 7 and 11 days after stress loading (31 and 34 days after the start of feeding, respectively) in the FLAB treatment group (Fig. 2B). These results suggested that FLAB may increase lacrimal fluid volume by affecting lacrimal fluid secretion.

Dry eye is a disease caused by decreased tear fluid retention due to decreased tear fluid secretion and leads to corneal cell damage. Compared to the normal group, the corneal epithelium layer in the stress loading group thinned down (Figs. 3A, B). However, the defects in corneal epithelium thickness after stress loading were not significantly improved in the FLAB-fed group (Figs. 3A, B), which suggested that the lacrimal secretion improved by FLAB treatment is not sufficient for ameliorating the corneal damage induced by stress loading.

The Effects of FLAB on Hippocampal *Bdnf* Expression in a Stress-Induced Dry Eye Model To elucidate the mechanisms underlying the FLAB-mediated enhancement of lacrimal secretion, we assessed *Bdnf* expression in the brain,

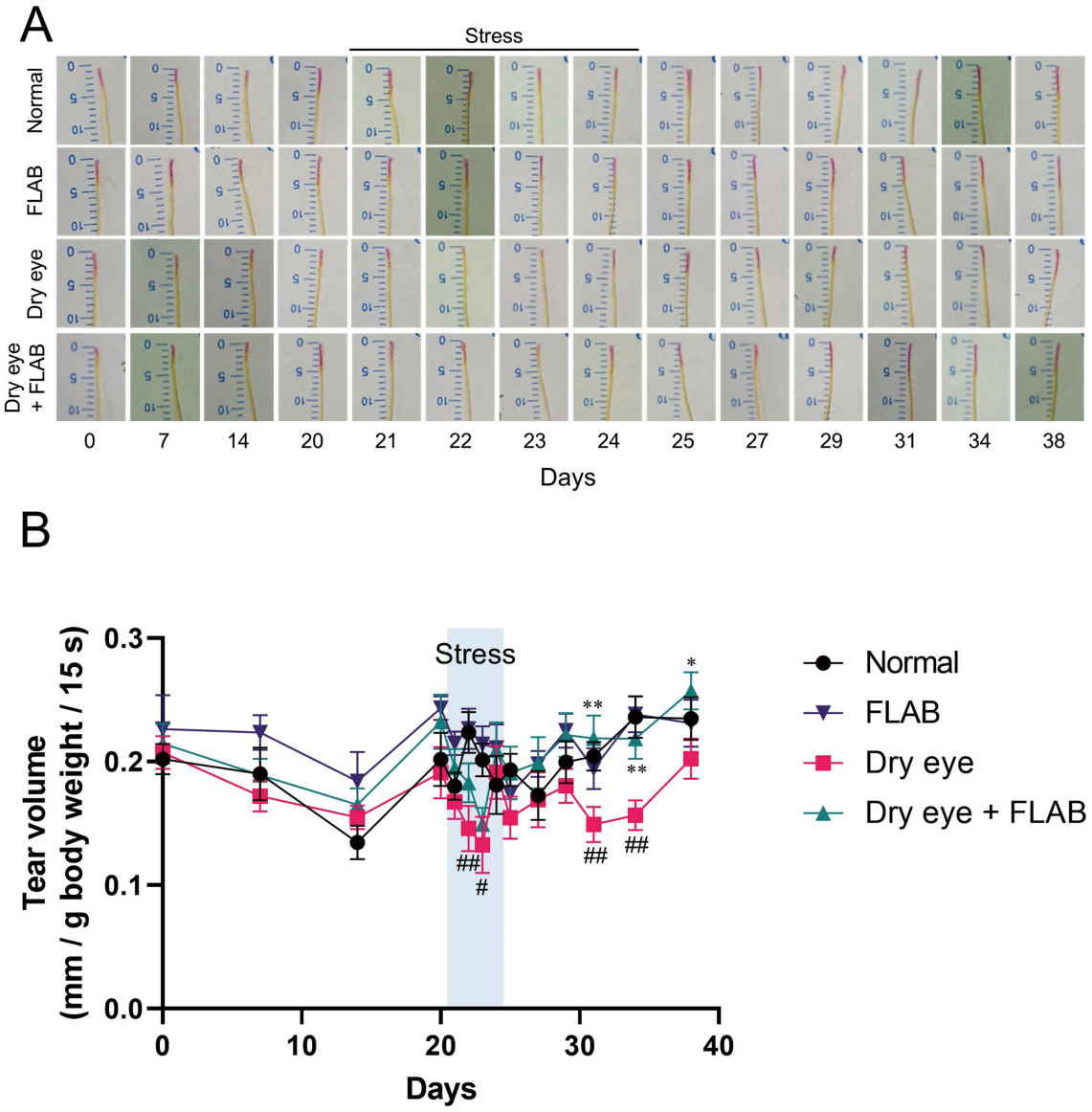

Fig. 2. FLAB Intake Prevented the Decrease in Tear Fluid Volume in a Stress-Induced Dry Eye Model

(A) Representative images of cotton phenol red threads and (B) quantification of tear volumes in each group [Normal, AIN-93M diet without stress loading (black); FLAB, AIN-93M diet containing 3% FLAB without stress loading (purple); Dry eye, AIN-93M diet with stress loading (red); and Dry eye + FLAB, AIN-93M diet containing 3% FLAB with stress loading (green)]. The data comprise the means ± SEMs (n = 6-10). #$p < 0.05$, ##$p < 0.01$ vs. Normal group, *$p < 0.05$, **$p < 0.01$ vs. Dry eye group (Student's *t*-test).

which has been implicated in regulating tear fluid secretion. In a previous report,[15] the expression levels of *Bdnf* transcripts (isoforms 1 and 2) were reported to be reduced in the hippocampus of stress loaded animals. On the last day of the study (day 38 after the start of feeding), the RNAs in the hippocampus were extracted for the qRT-PCR experiments. We measured the most abundant brain *Bdnf* transcripts (isoforms 1, 2, 4, and 6). Total *Bdnf* levels were assessed by the primer pairs in exon 9, which is common to all the isoforms (Table 1). The expression of total *Bdnf* (Fig. 4A), *Bdnf* isoform 2 (Fig. 4C), and *Bdnf* isoform 6 (Fig. 4E) were elevated in the FLAB treatment group, but *Bdnf* isoform 1 (Fig. 4B) was not. On the contrary, FLAB intake tended to decline the level of *Bdnf* isoform 4, and significantly decreased it after stress loading (Fig. 4D). Taken together, the intake of FLAB increases the expression levels of specific *Bdnf* isoforms in the murine hippocampus.

The Effects of FLAB on Plasma IL-10 Levels in a Stress-Induced Dry Eye Model Specific lactic acid bacteria are known to activate M2 macrophages and stimulate the production of inhibitory cytokines, such as the anti-inflammatory cytokine IL-10.[17] On the last day of the study (day 38 after the start of feeding), blood was drawn from the mice, and plasma was harvested to determine the amount of IL-10 using an ELISA technique. The plasma levels of IL-10 increased in FLAB-fed mice (Fig. 5). This inhibitory cytokine production stimulated by FLAB intake may contribute to the protection of lacrimal secretion under stress conditions.

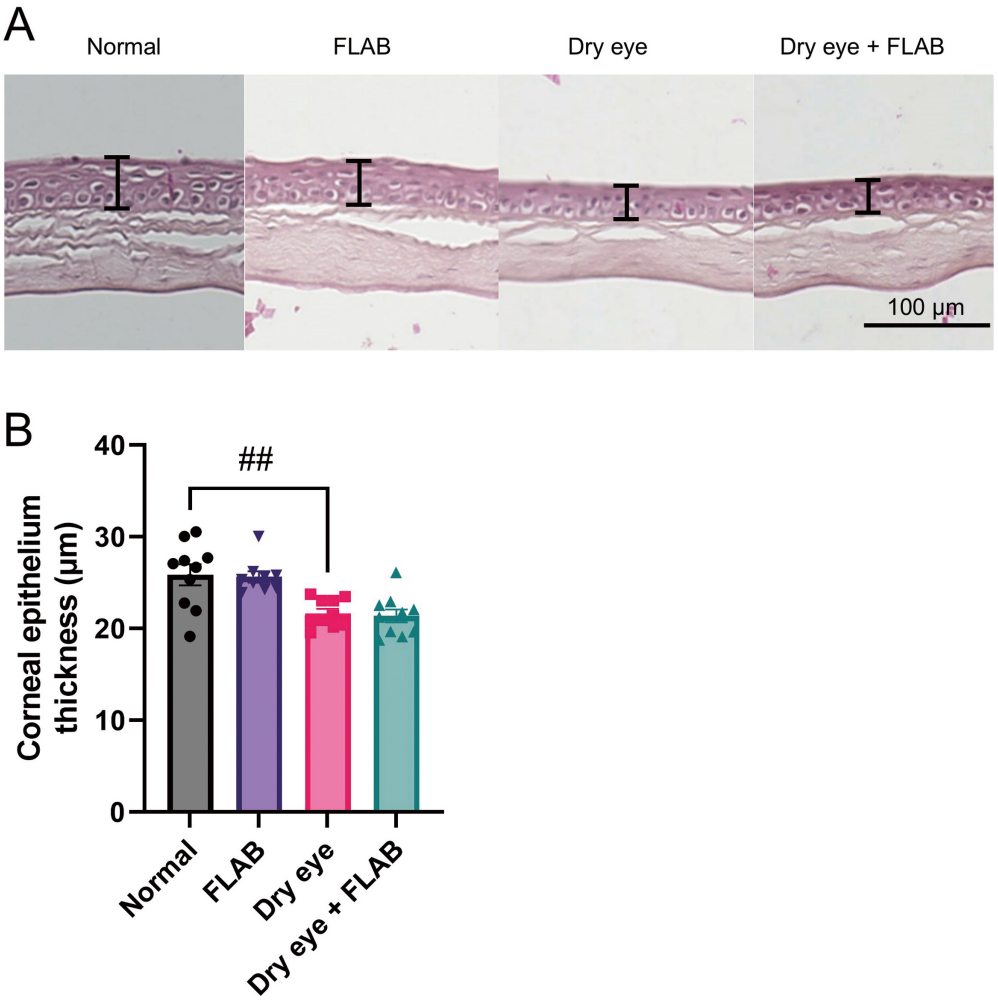

Fig. 3. FLAB Treatment Had No Significant Effects on Corneal Epithelium Thinning in a Stress-Induced Dry Eye Model

(A, B) The mouse groups comprise the following: Normal, AIN-93M diet without stress loading (black); FLAB, AIN-93M diet containing 3% FLAB without stress loading (purple); Dry eye, AIN-93M diet with stress loading (red); and Dry eye + FLAB, AIN-93M diet containing 3% FLAB with stress loading (green). (A) Representative images of hematoxylin and eosin staining of the corneal epithelium. The black brackets represent the measured corneal epithelium thicknesses and are shown in B. Bar = 100 μm. (B) Quantitative data of the corneal thickness. The data comprise the means ± SEMs (n = 9, 10). ##$p < 0.01$ vs. Normal group (Tukey's test).

DISCUSSION

In this study, we evaluated whether FLAB enhances lacrimal fluid secretion. The lacrimal fluid significantly decreased after stress loading, which improved in the FLAB treatment group (Fig. 2). Moreover, the mRNA levels of total *Bdnf*, and *Bdnf* isoforms 2 and 6 were elevated in the hippocampus of the FLAB treatment group (Fig. 4). Finally, FLAB also increased plasma IL-10 levels (Fig. 5). Taken together, the intake of dried FLAB can improve tear fluid volume in a stress loading mouse model.

Dry eye is a disease in which the stability of tear fluid decreases, causing ocular discomfort and abnormal visual function as well as damage to the ocular surface. Food ingredients with lacrimal fluid secretion effects are expected to prevent and improve dry eye. We used the stress-induced dry eye model in which stress loading is achieved by the combination of a psychological stressor (restraint) and a mechanical stressor (blow).[15] In this particular model, an enriched environment before or after stress loading can improve tear secretion.[15] In the current study, the decrease in lacrimal fluid after stress loading was improved in the FLAB treatment group between 7 and 11 days after stress loading (31 and 34 days after the start of feeding, respectively; Fig. 2B). However, there was a time lag before the effect was observed. Additionally, we found no significant improvement in corneal epithelium thickness with and without FLAB treatment (Figs. 3A, B). This suggested that the stress loading may be too severe to be protected by tear secretion. Therefore, further studies are required to assess whether FLAB can protect the ocular surface.

A *BDNF* polymorphism has been reported to be associated with dry eye diseases in humans.[18] Moreover, it has been suggested that BDNF is involved in tear fluid secretion.[15] It is also known that lactobacilli suppress the decrease in BDNF levels observed under conditions of chronic restraint stress.[19] Interestingly, we found that lactobacillus treatment increased *Bdnf* mRNA expression in the murine hippocampus. The amount of *Bdnf* expression in the hippocampus is more enhanced in the dry eye model mice treated with FLAB (Fig. 4). We found that FLAB promotes lacrimal fluid secretion. Thus, FLAB may be

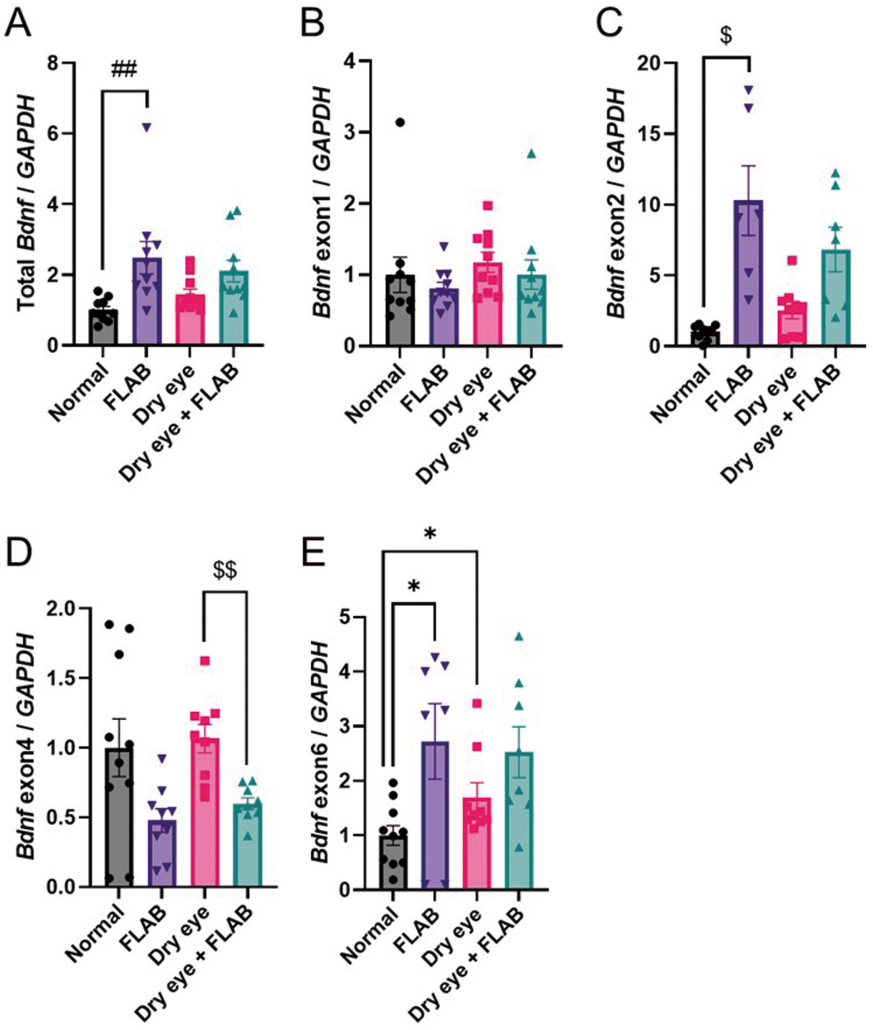

Fig. 4. Hippocampal *Bdnf* mRNA Expression Was Elevated in FLAB-Treated Mice

(A-E) The mouse groups comprise the following: Normal, AIN-93M diet without stress loading (black); FLAB, AIN-93M diet containing 3% FLAB without stress loading (purple); Dry eye, AIN-93M diet with stress loading (red); and Dry eye + FLAB, AIN-93M diet containing 3% FLAB with stress loading (green). The hippocampal *Bdnf* transcripts [isoform 1 (B), isoform 2 (C), isoform 4 (D), isoform 6 (E), and total (A)] were evaluated and are shown as the means ± SEMs (n = 6-10). ##$p < 0.01$ vs. Normal group (Tukey's test), $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ vs. Normal group or vs. Dry eye group (Games-Howell test), $*p < 0.05$ vs. Normal group (Student's *t*-test).

useful as a lacrimal fluid secretion stimulant, which may suppress eye fatigue and discomfort associated with reduced eye moisture. Moreover, FLAB may prevent dry eyes by maintaining eye moisture. These results suggested that lactobacilli increase tear fluid volume by affecting both the lacrimal secretion mechanism and inhibitory cytokine production. The decrease in tear fluid volume in the restrained blast dry eye mouse model was suppressed by lactobacilli intake. These results indicated that lactobacillus supplementation may be a strategy for the prevention and treatment of dry eye.

The plasma concentration of IL-10 is higher in the group treated with FLAB, the lacrimal fluid secretion stimulator (Fig. 5). Since IL-10 is an anti-inflammatory cytokine believed to suppress inflammatory responses, our results suggested that the increased IL-10 in plasma may contribute to the reduction of eye fatigue and discomfort caused by reduced tear fluid secretion. Several studies have shown that probiotics, which stimulate immune cells in the intestines, can increase plasma IL-10 levels.[20,21] Moreover, it has been reported that the expression of IL-10 was increased by treatment with plas-

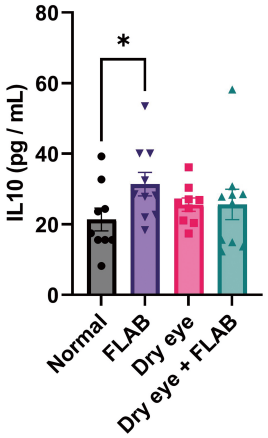

Fig. 5. The Plasma IL-10 Levels Were Increased in the FLAB-Treated Mice

The mice groups comprise the following: Normal, AIN-93M diet without stress loading (black); FLAB, AIN-93M diet containing 3% FLAB without stress loading (purple); Dry eye, AIN-93M diet with stress loading (red); and Dry eye + FLAB, AIN-93M diet containing 3% FLAB with stress loading (green). Plasma IL-10 levels in each group are shown as the means ± SEMs (n = 9-10). $*p < 0.05$ vs. Normal group (Student's *t*-test).

ma from mice fed *Lactobacillus pentosus TJ515*.[22] It remains unclear how probiotics can stimulate the production of IL-10. In the present study, we used dried powder forms of FLAB, which may impact microbiota directly. Further investigation is necessary to elucidate the mechanism underlying the effects of FLAB on both the immune system and lacrimal secretion.

Acknowledgments We thank Akihito Endo (Tokyo University of Agriculture) for providing *F. fructosus* FMO-85.

Author Contributions Conceptualization: E.S., W.O., T.Y., T.H., and M.S.; methodology, formal analysis, and investigation: E.S., W.O., T.Y., and M.S.; writing, reviewing, and editing: E.S., W.O., S.N., and M.S.; supervision: T.H., T.M., H.M., H.K., H.T., and M.S.

Abbreviations adhE, alcohol/acetaldehyde dehydrogenase gene; BDNF, brain-derived neurotrophic factor; FLAB, fructophilic lactic acid bacteria; IL-10, interleukin-10; LAB, lactic acid bacteria; PB, phosphate buffer; VDT, visual display terminal.

Conflicts of Interest This work was financially supported by API Co. Ltd. T.H., T.M., H.M., and H.K. are employees of API Co. Ltd.

REFERENCES

1) Uchino M, Schaumberg DA, Dogru M, Uchino Y, Fukagawa K, Shimmura S, Satoh T, Takebayashi T, Tsubota K. Prevalence of dry eye disease among Japanese visual display terminal users. *Ophthalmology*, **115**, 1982–1988 (2008).
2) Uchino M, Yokoi N, Uchino Y, Dogru M, Kawashima M, Komuro A, Sonomura Y, Kato H, Kinoshita S, Schaumberg DA, Tsubota K. Prevalence of dry eye disease and its risk factors in visual display terminal users: the Osaka study. *Am. J. Ophthalmol.*, **156**, 759–766 (2013).
3) Kojima T, Ibrahim OMA, Wakamatsu T, Tsuyama A, Ogawa J, Matsumoto Y, Dogru M, Tsubota K. The impact of contact lens wear and visual display terminal work on ocular surface and tear functions in office workers. *Am. J. Ophthalmol.*, **152**, 933–940.e2 (2011).
4) Bringer M-A, Gabrielle P-H, Bron AM, Creuzot-Garcher C, Acar N. The gut microbiota in retinal diseases. *Exp. Eye Res.*, **214**, 108867 (2022).
5) Jiao J, Yu H, Yao L, Li L, Yang X, Liu L. Recent Insights into the Role of Gut Microbiota in Diabetic Retinopathy. *J. Inflamm. Res.*, **14**, 6929–6938 (2021).
6) Gong H, Zhang S, Li Q, Zuo C, Gao X, Zheng B, Lin M. Gut microbiota compositional profile and serum metabolic phenotype in patients with primary open-angle glaucoma. *Exp. Eye Res.*, **191**, 107921 (2020).
7) Lin P, McClintic SM, Nadeem U, Skondra D. A Review of the Role of the Intestinal Microbiota in Age-Related Macular Degeneration. *J. Clin. Med.*, **10**, 2072 (2021).
8) Vinderola G, Ouwehand A, Salminen S, von Wright A. *Lactic Acid Bacteria: Microbiological and Functional Aspects*. CRC Press, (2019).
9) Lee K, Jeong JW, Shim JJ, Hong HS, Kim JY, Lee JL. *Lactobacillus fermentum* HY7302 Improves Dry Eye Symptoms in a Mouse Model of Benzalkonium Chloride-Induced Eye Dysfunction and Human Conjunctiva Epithelial Cells. *Int. J. Mol. Sci.*, **24**, 10378 (2023).
10) Endo A, Futagawa-Endo Y, Dicks LMT. Isolation and characterization of fructophilic lactic acid bacteria from fructose-rich niches. *Syst. Appl. Microbiol.*, **32**, 593–600 (2009).
11) Endo A, Maeno S, Tanizawa Y, Kneifel W, Arita M, Dicks L, Salminen S. Fructophilic Lactic Acid Bacteria, a Unique Group of Fructose-Fermenting Microbes. *Appl. Environ. Microbiol.*, **84**, e01290-18 (2018).
12) Endo A, Salminen S. Honeybees and beehives are rich sources for fructophilic lactic acid bacteria. *Syst. Appl. Microbiol.*, **36**, 444–448 (2013).
13) Maeno S, Tanizawa Y, Kanesaki Y, Kubota E, Kumar H, Dicks L, Salminen S, Nakagawa J, Arita M, Endo A. Genomic characterization of a fructophilic bee symbiont Lactobacillus kunkeei reveals its niche-specific adaptation. *Syst. Appl. Microbiol.*, **39**, 516–526 (2016).
14) De Simone N, Rocchetti MT, la Gatta B, Spano G, Drider D, Capozzi V, Russo P, Fiocco D. Antimicrobial Properties, Functional Characterisation and Application of Fructobacillus fructosus and Lactiplantibacillus plantarum Isolated from Artisanal Honey. *Probiotics Antimicrob. Proteins*, **15**, 1406–1423 (2023).
15) Sano K, Kawashima M, Imada T, Suzuki T, Nakamura S, Mimura M, Tanaka KF, Tsubota K. Enriched environment alleviates stress-induced dry-eye through the BDNF axis. *Sci. Rep.*, **9**, 3422 (2019).
16) Nishinaka A, Tanaka M, Aoshima K, Kuriyama A, Sasaki T, Otsu W, Yasuda H, Nakamura S, Shimazawa M, Hara H. The pathological association between the anterior eye segment and the retina in a murine model of neovascular glaucoma. *FASEB J.*, **36**, e22323 (2022).
17) Morita Y, Miwa Y, Jounai K, Fujiwara D, Kurihara T, Kanauchi O. *Lactobacillus paracasei* KW3110 Prevents Blue Light-Induced Inflammation and Degeneration in the Retina. *Nutrients*, **10**, 1991 (2018).
18) Hallak JA, Tibrewal S, Mohindra N, Gao X, Jain S. Single Nucleotide Polymorphisms in the BDNF, VDR, and DNASE 1 Genes in Dry Eye Disease Patients: A Case-Control Study. *Invest. Ophthalmol. Vis. Sci.*, **56**, 5990–5996 (2015).
19) Liang S, Wang T, Hu X, Luo J, Li W, Wu X, Duan Y, Jin F. Administration of Lactobacillus helveticus NS8 improves behavioral, cognitive, and biochemical aberrations caused by chronic restraint stress. *Neuroscience*, **310**, 561–577 (2015).
20) Sierra S, Lara-Villoslada F, Sempere L, Olivares M, Boza J, Xaus J. Intestinal and immunological effects of daily oral administration of Lactobacillus salivarius CECT5713 to healthy adults. *Anaerobe*, **16**, 195–200 (2010).
21) Gardlik R, Palffy R, Celec P. Recombinant probiotic therapy in experimental colitis in mice. *Folia Biol. (Praha)*, **58**, 238–245 (2012).
22) Hidaka Y, Kuriyama A, Nishinaka A, Takahashi K, Nakaya S, Kubota T. Nakamura Shinsuke and Shimazawa M, Hara H. Intake of Lactobacillus Pentosus TJ515 Prevents the Formation of Retinal Edema in Retinal Vein Occlusion Model Mice. *BPB Reports*, **6**, 47–54 (2023).