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ORIGINAL RESEARCH ARTICLE

Daily intake of *Citrus jabara* fruit peel powder (Japanese Patent No. 5,323,127) improves allergy-like symptoms: A randomized double-blind parallel-group comparative study

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ABSTRACT

Citrus jabara (CJ) is a rare citrus fruit that used to grow naturally only in the southern part of the Kii Peninsula in Japan. Human intervention studies with oral intake of CJ fruit have shown its anti-allergic effects, but the testing method was a pre-post comparison study. In this study, we conducted a randomized, double-blind, parallel-group interventional study to evaluate the volume-dependent effects of oral intake of CJ fruit peel powder (Japanese Patent No. 5,323,127) on nasal and eye allergy-like symptoms. Ninety healthy adults were allocated to three groups and given test foods containing 1,000, 500, and 0 mg of CJ peel powder, with one packet per day for 4 weeks. After excluding those who dropped out or deviated from the study protocol, 73 were included in the efficacy analysis and 86 in the safety analysis. The high-dose group (1,000 mg/day) was significantly lower than the placebo group in the scores of "nasal and eye symptoms" at week 4, and "blocked nose" at weeks 2 and 4 in the evaluation of question I of Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ No. 1). The changes in scores (difference from the pre-observation period) on the Nasal and Eye Symptom Questionnaire showed a dose-dependent reduction in rhinorrhea. In the safety evaluation, there were no significant differences in examinations of physiology, hematology, and blood biochemistry between the groups, and no adverse events attributable to the test foods were observed. These results suggest that intake of CJ peel powder can alleviate allergy-like symptoms.

Keywords: *Citrus jabara* Peel Powder; Allergy-like Symptoms; Randomized-double-blind-parallel-group Interventional Study; Foods with Function Claims

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1. Introduction

In the epidemiological Survey of Allergic Rhinitis in Japan 2019, the prevalence of allergic rhinitis in our country was 49.2%, pollinosis (including Japanese cedar and other pollinosis) was 38.8%, and perennial allergic rhinitis was 24.5%, showing an increase in prevalence compared to past surveys[1]. Meanwhile, in an effort to curb medical costs, the Federation of Health Insurance Associations has proposed that medical hay fever treatments similar to over-the-counter (OTC) drugs be excluded from insurance coverage.

In addition, attention to “ME-BYO” and “preventive medicine” that focuses on prevention of disease and serious illness is increasing for reasons of both cost and quality of life (QOL). Reflecting this situation, “allergies” were added to the health claims of “Foods with Function Claims” under the jurisdiction of the Consumer Affairs Agency in spring 2019.

Citrus jabara (CJ) is a rare citrus fruit that used to grow naturally only in the southern part of the Kii Peninsula in Japan, and its fruits been reported to have anti-allergic and anti-inflammatory effects[2-7]. Some human intervention studies with oral intake of CJ fruits have evaluated the anti-allergic effects of fruit juice[6] and fermented product[7], but the test methods were before-and-after comparison studies.

These anti-allergic and anti-inflammatory effects are attributed, at least in part, to the flavonoid narirutin, which is abundantly contained in CJ fruit. Narirutin has been reported to inhibit increases in eosinophils and blood immunoglobulin (Ig) E in mouse models of asthma[8] and to suppress inflammation[9].

We focused on the fact that narirutin is unevenly distributed in CJ fruit, with the vast majority (about 90%) in the peel rather than in the juice, which has been conventionally used. Furthermore, the safety of CJ fruit peels has improved, with the development of a CJ fruit peel powder with high narirutin content and proven safety[2,10].

In the present study, we report the results of a randomized, double-blind, parallel-group, comparative interventional study on the volume-dependent effects of oral intake of CJ peel powder[10] on nasal and eye allergy-like symptoms.

2. Experimental

2.1 Participants

The participants were healthy Japanese men and women between the ages of 20 and 65 with subjective symptoms of eye and nose discomfort (sneezing, runny nose, nasal congestion, itchy eyes, etc.) in daily life. Ninety participants were selected based on the results of blood tests and medical interviews.

The exclusion criteria for the selection of participants were as follows: (1) those with severe or worse allergic rhinitis symptoms, (2) those with acute rhinitis, sinusitis, nasal polyps, hypertrophic rhinitis, or deviated nasal septum, (3) those with bronchial asthma, (4) those with serious liver, heart, kidney, respiratory, endocrine, or metabolic diseases, (5) those who were undergoing or had undergone specific desensitization therapy, (6) those who were receiving any medication for treatment, (7) those who had current or previous drug allergies or food allergies, (8) those who had a history of discomfort or problems with physical symptoms after eating citrus fruits, (9) those who routinely consumed “Foods for specific health uses” or “Foods with Function Claims” (however, this did not apply to those who are able to suspend their intake during the study period at the time of obtaining consent), (10) those who were pregnant, lactating, or who wished to become pregnant during the study, (11) those who had experienced sickness or deterioration of physical condition due to blood collection in the past, or those who had been told that their blood vessels are too small to facilitate blood collection, (12) those who had participated or were currently participating in other clinical trials within one month prior to obtaining consent, or those who planned to thus participate during the study, (13) those who might change their lifestyle during the study, such as taking a long trip, (14) heavy alcohol drinkers (60 g/day in alcohol equivalent), (15) those with extremely irregular dietary habits and irregular life rhythms, such as those who work in shifts or late at night, and (16) others who were judged by the responsible medical doctor to be unsuitable as subjects for this study.

This study was conducted under the ethical review and approval of the Clinical Trial Review Committee of Hakusui-Kai Suda Clinic Medical Corporation (approved on January 26, 2021, approval number: 2021-004), in compliance with the “Helsinki Declaration” and the “Ethical Guidelines for Medical Research Involving Human Subjects” (Ministry of Education, Culture,

Sports, Science and Technology (MEXT), and the Ministry of Health, Labor and Welfare (MHLW)). The study was conducted under the supervision of a physician and with the cooperation of a third-party CRO to ensure the human rights and safety of the participants and the reliability of the study data. The study protocol for this study was registered in advance with the University Hospital Medical Information Network (UMIN) (UMIN000043224).

2.2 Test foods

The CJ peel powder[10] used in this study was produced by Jabara Laboratory Co., Ltd. This powder was standardized to contain at least 75 mg/g of narirutin and 70 μg/g of chlorophyll a and b. Maltodextrin, which does not affect eye and nasal health functions, was used for the placebo food. The test foods containing CJ peel powder, i.e., the high-dose test food consisting of 1,000 mg of CJ peel powder and 1,000 mg of reduced maltose, the low-dose test food consisting of 500 mg of CJ peel powder and 1,500 mg of reduced maltose, and the placebo food consisting of 1,000 mg of maltodextrin and 1,000 mg of reduced maltose, were manufactured by Asunaro Institute Chemical Co., Ltd. These foods were packaged in aluminum pouches and were visually indistinguishable.

2.3 Study design

The study was conducted in a randomized, double-blind, parallel-group trial under the supervision of a physician. Participants were given a full explanation of the study, and written consent was obtained from all participants. Randomization was performed by Contract Research Organization (CRO)-affiliated personnel not directly involved in the study, using Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ) question I, nasal remarks scores, and scores on the Nasal and Eye Symptom Questionnaire as adjustment factors. All groups were asked to take one packet a day with water or lukewarm water every day before breakfast for four weeks, and to record whether or not they took the packet in an electronic diary. The intake period of the test and placebo foods was conducted from May 2021 to June 2021.

During the study, the intake of health foods and supplements as well as foods with anti-allergic effects, such as Tencha (sugar beet), was prohibited. Except in emergencies, drugs were to be used only with the permission of the investigator, and when used, the reason for use, name of drug used, amount used, duration of use, etc. were to be entered in the electronic diary and the investigator was to be notified.

During the study, the participants were expected to lead the same lifestyle as before participating in the study. In particular, binge drinking, excessive dietary restrictions, changes in eating habits due to overseas travel, changes in exercise habits, lack of sleep due to excessive late nights, or changes in drinking habits were not allowed. Participants who significantly violated these compliance requirements were excluded from the study. In addition, participants who skipped the test foods more than 3 days during the test periods were also excluded.

2.4 Endpoint

2.4.1 Japanese Rhino-conjunctivitis Quality of Life Questionnaire (JRQLQ No. 1)

The scores of nasal and eye symptoms (runny nose, sneezing, blocked nose, itchy nose, itchy eyes, watery eyes) in the JRQLQ No. 1 were evaluated as the primary endpoint, and the total score of the JRQLQ No. 1 was evaluated as the secondary endpoint. The participants were asked to record the results in a questionnaire at screening and at the end of weeks 2 and 4 of the study.

2.4.2 Evaluation of Nasal Remarks Score

At the time of screening and the end of the study (at week 4), following 4 endpoints such as “swelling of concha nasalis inferior mucosa”, “color of concha nasalis inferior mucosa”, “aqueous secretion”, and “character of nasal mucus” were evaluated by an otolaryngologist on a 4-point scale, according to the practical guideline for the management of allergic rhinitis in Japan[11].

2.4.3 Nasal and Eye Symptom Questionnaire

At the end of each day during the study, the participants were asked to evaluate and record scores for "paroxysmal sneezing", "rhinorrhea", "nasal blockage" "itchy eyes", and "watery eyes" on a 5-point scale[12]. The scores for each endpoint in the diary questionnaire were summed and compared for the two weeks of the pre-observation period, the first half, and the second half of the study.

2.5 Physical examination and blood tests

In the physical examination, height, weight, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse rate (P) were measured. Hematological tests included white blood cell count, red blood cell count (WBC), platelet count (RBC), hemoglobin (Hb), and hematocrit (HCT). Biochemical blood analysis included values for total protein (TP), total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), total bilirubin (T-Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (γ-GTP), creatine phosphokinase (CPK), fasting blood glucose (GLU), and hemoglobin A1c (HbA1c). Serum immunoglobulin E (IgE) specific for house dust, *Dermatophagoides pteronyssinus*, Japanese cedar pollen, and Japanese cypress pollen were measured. Of the above endpoints, height, HbA1c, and specific IgE were measured only at the screening, while all other clinical test items were measured at the screening and at the end of the study (week 4).

2.6 Statistical analysis

Statistical analysis was performed using the computer software "IBM SPSS Statistics Subscription". Each endpoint was presented as a mean ± standard deviation (SD). A two-tailed test was used to determine the significance probability, with "significant difference" determined when the significance level was less than 5%, and "trend" when the significance level was between 5% and 10%.

For the comparison before and after the intake of the test or placebo foods, normality was first tested by the Shapiro-Wilk test. The paired *t*-test (PTT) was used when normality could be assumed, and the Wilcoxon signed-rank test (PWT) was used when normality could not be assumed. For between-group comparisons of the low-dose, high-dose, and placebo groups, the Tukey-Kramer test (TK) was performed when normality and homoscedasticity could be assumed, and the Kruskal-Wallis test followed by Bonferroni correction (KWB) was performed when normality could not be assumed. The same was done for the comparison of the amount of change.

The analysis for efficacy was based on the participants who completed the study, excluding those who met the exclusion criteria. For the safety analysis, all participants were included in the study.

3. Results

3.1 Participants

The flow of participant selection is shown in **Figure 1**. In this study, 182 candidates were recruited, 90 participants started taking the test foods, and 86 completed the prescribed schedule. The reasons for the dropout of the other 4 participants were confirmed to be unrelated to the study procedures or their effects. Of the 86 participants, 13 deviated from the study protocol, leaving 73 participants in the efficacy analysis and 86 in the safety analysis. Reasons for dropout or exclusion from the analysis are shown in **Table 1**, and participant background is shown in **Table 2**. There were no significant differences between groups in gender, age, height, weight, BMI, SBP, DBP, P, Hb1Ac, and "nasal and nose symptoms" in JRQLQ No. 1. The number of participants using allergy medication in this study was 10 out of 90 at the pre-observation period, 1 out of 90 at week 2, and 1 out of 86 at week 4. The drug scores of the allergy medi-

cation users were in the mild range. These results indicate that more than half of participants were in the healthy range.

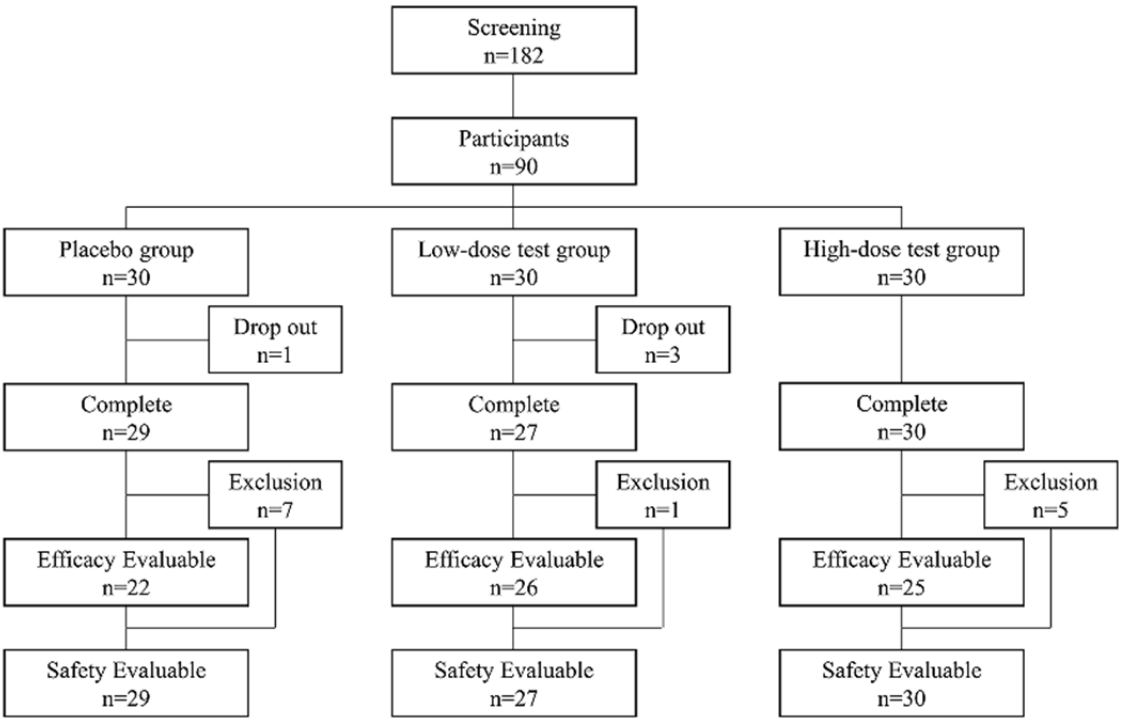

Figure 1. The flow of participant selection and evaluation.

Table 1. Reason for exclusion for participants

ID	Reason for exclusion
8	Noncompliance with restrictions
14	Withdrawal of consent
16	Noncompliance with restrictions
23	Noncompliance with restrictions
37	Noncompliance with restrictions
39	Less than 90% intake of test foods
40	Noncompliance with restrictions
41	Noncompliance with restrictions
45	Noncompliance with restrictions
54	Noncompliance with restrictions
66	Noncompliance with restrictions
67	Withdrawal of consent
71	Noncompliance with restrictions
82	Noncompliance with restrictions
83	Withdrawal of consent
85	Noncompliance with restrictions
89	Withdrawal of consent

Table 2. Participant background

	Placebo	Low-dose	High-dose
Number	30 (M: 13, F: 17)	30 (M: 14, F: 16)	30 (M: 14, F 16)
Age (years)	44.4 ± 12.0	44.9 ± 12.7	44.7 ± 11.4
Height (cm)	162.8 ± 8.1	164.4 ± 7.3	163.5 ± 8.6
Weight (kg)	59.2 ± 10.1	60.2 ± 11.0	58.6 ± 10.3
BMI	22.2 ± 3.0	22.2 ± 3.1	21.8 ± 2.5
SBP (mmHg)	112.8 ± 13.2	112.9 ± 14.2	115.1 ± 12.8
DPB (mmHg)	75.3 ± 8.7	74.5 ± 10.9	77.6 ± 8.2
P (pbm)	72.2 ± 9.1	72.0 ± 8.7	72.8 ± 12.5
HbA1c	5.34 ± 0.29	5.27 ± 0.33	5.23 ± 0.28
Nasal and eye symptom score in the question I of JRQLQ No. 1	12.1 ± 3.6	12.4 ± 3.8	12.3 ± 3.9

Values are shown as means ± SDs.

3.2 JRQLQ No. 1

The results of Question I of the JRQLQ No. 1 are shown in **Table 3**. There were no significant differences among the low-dose, high-dose, and placebo groups in scores for each endpoint or "nasal and eye symptoms" in the preliminary screening. In the between-group comparison at week 2, the high-dose group was significantly lower than the placebo group in the score for "blocked nose". At week 4, the high-dose group had significantly lower scores than the placebo group for "blocked nose" and "nasal and eye symptoms", and the low-dose group tended to have lower scores than the placebo group for "sneezing". There were no significant differences in comparisons of other endpoints and in the amount of change in scores. In the before-and-after comparison, scores at weeks 2 and 4 were significantly lower than those at screening for all endpoints in the low-dose, high-dose, and placebo groups.

The total score of JRQLQ No. 1 tended to be lower in the high-dose group than in the placebo group at week 2. There were no significant differences between groups at other time points or in comparisons of the amount of change in scores. In the before-and-after comparison, scores at weeks 2 and 4 were significantly lower than at screening for all endpoints in the low-dose, high-dose, and placebo groups.

Table 3. Results of JRQLQ No. 1

Parameter	Group	Score			Amount of change in score	
		Screening	Week 2	Week 4	Week 2 – SCR	Week 4 – SCR
Nasal and eye symptoms	Placebo	12.1 ± 3.7	5.8 ± 2.8***	5.1 ± 2.9***	–6.3 ± 3.6	–7.0 ± 4.0
	Low-dose	12.1 ± 3.4	4.7 ± 2.5***	3.8 ± 1.9***	–7.4 ± 3.2	–8.3 ± 3.2
	High-dose	11.6 ± 3.5	4.2 ± 2.0***	3.2 ± 1.9$^{***\#\#}$	–7.4 ± 4.2	–8.4 ± 3.9
Runny nose	Placebo	2.3 ± 0.9	1.0 ± 0.7***	0.8 ± 0.9***	–1.4 ± 0.7	–1.5 ± 1.0
	Low-dose	2.2 ± 1.0	0.8 ± 0.5***	0.7 ± 0.5***	–1.5 ± 1.0	–1.5 ± 1.0
	High-dose	2.1 ± 0.9	0.8 ± 0.5***	0.6 ± 0.6***	–1.4 ± 1.0	–1.5 ± 1.0
Sneezing	Placebo	2.1 ± 0.9	1.2 ± 0.8***	1.3 ± 0.9***	–0.9 ± 1.0	–0.8 ± 1.0
	Low-dose	2.0 ± 0.9	1.0 ± 0.5***	0.8 ± 0.4$^{***\#}$	–1.0 ± 1.0	–1.1 ± 0.9
	High-dose	1.9 ± 0.8	1.0 ± 0.4***	1.0 ± 0.4***	–0.8 ± 0.8	–0.9 ± 0.9
Blocked nose	Placebo	2.0 ± 1.2	1.1 ± 0.9***	0.9 ± 0.6***	–1.0 ± 1.0	–1.2 ± 1.0
	Low-dose	1.9 ± 0.9	0.8 ± 0.6***	0.7 ± 0.6***	–1.1 ± 0.8	–1.2 ± 0.9
	High-dose	2.0 ± 0.8	0.6 ± 0.6$^{***\#\#}$	0.4 ± 0.6$^{***\#\#}$	–1.4 ± 1.1	–1.5 ± 1.0
Itchy nose	Placebo	1.8 ± 0.9	0.7 ± 0.6***	0.7 ± 0.8***	–1.1 ± 0.9	–1.1 ± 0.8
	Low-dose	1.7 ± 1.0	0.7 ± 0.8***	0.5 ± 0.6***	–0.9 ± 1.1	–1.1 ± 1.0
	High-dose	1.6 ± 0.7	0.4 ± 0.5***	0.3 ± 0.5***	–1.2 ± 0.8	–1.3 ± 0.9
Itchy eyes	Placebo	2.5 ± 0.9	1.1 ± 0.7***	0.9 ± 0.6***	–1.4 ± 1.0	–1.6 ± 1.0
	Low-dose	2.6 ± 0.9	0.8 ± 0.7***	0.7 ± 0.7***	–1.8 ± 1.0	–1.9 ± 0.9
	High-dose	2.5 ± 0.8	0.8 ± 0.5***	0.5 ± 0.5***	–1.7 ± 1.0	–2.0 ± 0.9
Watery eyes	Placebo	1.5 ± 1.1	0.8 ± 0.8**	0.6 ± 0.6***	–0.7 ± 1.4	–0.8 ± 1.2
	Low-dose	1.7 ± 0.9	0.6 ± 0.5***	0.3 ± 0.5***	–1.2 ± 0.9	–1.4 ± 0.9
	High-dose	1.5 ± 0.9	0.6 ± 0.6***	0.4 ± 0.5***	–1.0 ± 1.0	–1.1 ± 0.9
Total score of the JRQLQ No. 1	Placebo	40.2 ± 18.7	14.9 ± 10.4***	12.3 ± 11.6***	–25.3 ± 16.9	–27.9 ± 20.0
	Low-dose	37.3 ± 13.8	12.0 ± 8.7***	9.0 ± 6.4***	–25.4 ± 14.4	–28.3 ± 13.9
	High-dose	38.8 ± 16.9	9.7 ± 7.9$^{***\#}$	7.6 ± 6.4***	–29.1 ± 18.6	–31.2 ± 18.0

Differences in individual scores at screening and after intake were evaluated within groups by PTT or PWT, and between groups by TK or KWB. **, significant to screening (p <0.05); ***, significant to screening (p <0.01); $^{\#}$, significant trend to placebo (p <0.1); $^{\#\#}$, significant to placebo (p <0.05). Values are shown as means ± SDs.

3.3 Evaluation of Nasal Remarks Score

The results of the evaluation of nasal signs are shown in **Table 4**. In the comparison between groups, "swelling of concha nasalis inferior mucosa" at week 4 was significantly lower in the low-dose group than in the placebo group, and also tended to be lower in the high-dose group.

Table 4. Evaluation of nasal remarks score

Parameter	Group	Score		Amount of change in score
		Screening	Week 4	Week 4 - SCR
Swelling of concha nasalis inferior mucosa	Placebo	2.6 ± 1.0	2.6 ± 0.8	0.0 ± 0.7
	Low-dose	2.2 ± 0.9	2.0 ± 0.5$^{*\#\#}$	–0.3 ± 0.8
	High-dose	2.7 ± 1.0	2.4 ± 0.9**	–0.3 ± 0.6
Color of concha nasalis inferior mucosa	Placebo	2.4 ± 1.1	2.0 ± 0.7	–0.3 ± 1.1
	Low-dose	2.2 ± 0.8	1.8 ± 0.4***	–0.4 ± 0.7
	High-dose	2.5 ± 0.9	1.9 ± 0.4***	–0.6 ± 1.0
Watery secretions	Placebo	2.2 ± 0.8	1.8 ± 0.5**	–0.4 ± 0.7
	Low-dose	1.9 ± 0.7	1.7 ± 0.5	–0.2 ± 0.8
	High-dose	2.1 ± 0.9	1.6 ± 0.5**	–0.5 ± 0.8
Character of nasal mucus	Placebo	3.2 ± 1.1	2.8 ± 1.1	–0.4 ± 1.3
	Low-dose	2.6 ± 1.2	2.3 ± 1.0	–0.2 ± 1.5
	High-dose	2.8 ± 1.3	2.3 ± 1.1**	–0.6 ± 1.2

Differences in individual scores at screening and after intake were evaluated within groups by PWT, and between groups by KWB. *, tend to screening (p <0.1); **, significant to screening (p <0.05); ***, significant to screening (p <0.01); $^{\#\#}$, significant to placebo (p <0.05). Values are shown as mean ± SD.

On the other hand, there was no significant difference in "color of concha nasalis inferior mucosa", "aqueous secretion", or "character of nasal mucus" among the low-dose, high-dose, and placebo groups.

In the comparison of the changes in scores (differences from screening), there were no significant differences among the groups for changes in "swelling of concha nasalis inferior mucosa", "color of concha nasalis inferior mucosa", "aqueous secretion", and "character of nasal mucus".

In the before-and-after comparison, "swelling of concha nasalis inferior mucosa" showed a decreasing trend in the low-dose group and a significant decrease in the high-dose group at week 4 compared to the screening. The "color of concha nasalis inferior mucosa" score was significantly lower in the low-dose and high-dose groups at week 4 compared to the screening. For "aqueous secretion", the high-dose and placebo groups had significantly lower scores at week 4 than at the screening, with no significant change in the low-dose group. In character of nasal mucus, the high-dose group showed a significantly decreased score at week 4 compared to the screening, while the low-dose and placebo groups showed no significant change.

3.4 Nasal and Eye Symptoms Questionnaire

The results of the Nasal and Eye Symptoms Questionnaire are shown in **Table 5**. In the comparison of the scores of each endpoint on the nasal and eyes symptom questionnaire, there was no significant difference among the low-dose, high-dose and placebo groups for any of the following two weeks: pre-observation period, and the first and the second half of the study period.

In the comparison of changes in scores among the 3 groups (difference from the pre-observation period), the reduction in "paroxysmal sneezing" was significantly greater in the low-dose group than in the placebo group, for both the first half and second half of the study period. The reduction in "rhinorrhea" was significantly greater in the high-dose than the placebo group in both the first and second half of the study period; in addition, it was significantly greater in the low-dose than the placebo group in the first half of the study period and tended to be greater in the second half of the study period. The reduction in "itchy eyes" tended to be greater in the low-dose group than in the placebo group in the first half of the study period.

Table 5. Nasal and Eye Symptoms Questionnaire

Parameter	Group	Score			Amount of change in score	
		Pre observation	First half	Second half	First half - pre	Second half - pre
Paroxysmal sneezing	Placebo	19.2 ± 8.9	12.6 ± 9.0***	12.4 ± 8.8***	–6.5 ± 7.0	–6.8 ± 8.8
	Low-dose	21.6 ± 7.9	9.3 ± 6.8***	8.4 ± 6.8***	–12.3 ± 7.6$^{\#\#}$	–13.2 ± 9.0$^{\#\#}$
	High-dose	23.0 ± 8.3	11.8 ± 6.0***	11.0 ± 5.2***	–11.2 ± 8.8	–12.0 ± 8.5
Rhinorrhea	Placebo	22.6 ± 11.0	15.4 ± 8.9***	14.4 ± 8.8***	–7.2 ± 8.3	–8.2 ± 9.3
	Low-dose	23.9 ± 9.9	11.3 ± 8.7***	10.3 ± 8.2***	–12.7 ± 6.2$^{\#\#}$	–13.6 ± 7.0$^{\#}$
	High-dose	24.3 ± 9.5	11.6 ± 6.6***	9.8 ± 6.5***	–12.7 ± 8.2$^{\#\#}$	–14.4 ± 8.4$^{\#\#}$
Nasal blockage	Placebo	17.6 ± 11.1	9.5 ± 7.6***	7.3 ± 6.8***	–8.2 ± 7.4	–10.4 ± 9.1
	Low-dose	19.6 ± 9.2	7.2 ± 8.1***	5.5 ± 7.2***	–12.4 ± 8.3	–14.1 ± 8.7
	High-dose	17.3 ± 8.1	5.6 ± 6.7***	4.2 ± 5.6***	–11.7 ± 8.0	–13.1 ± 7.9
Itchy eyes	Placebo	22.0 ± 9.6	12.6 ± 10.2***	9.2 ± 9.5***	–9.5 ± 8.3	–12.8 ± 9.2
	Low-dose	24.8 ± 9.9	8.6 ± 8.6***	8.0 ± 9.4***	–16.2 ± 8.9$^{\#}$	–16.7 ± 9.1
	High-dose	23.0 ± 10.7	8.6 ± 8.3***	5.8 ± 6.2***	–14.4 ± 10.9	–17.2 ± 10.6
Watery eyes	Placebo	14.8 ± 9.3	7.8 ± 8.4***	4.4 ± 6.2***	–7.0 ± 7.0	–10.4 ± 7.5
	Low-dose	17.2 ± 11.6	4.3 ± 5.0***	3.6 ± 5.6***	–12.9 ± 10.8	–13.6 ± 10.5
	High-dose	15.7 ± 10.4	4.4 ± 6.0***	2.9 ± 4.2***	–11.4 ± 8.6	–12.8 ± 10.0

Differences in individual scores at screening and after intake were evaluated within groups by PTT or PWT, and between groups by TK or KWB. *, significant trend to screening (p <0.1); **, significant to screening (p <0.05); ***, significant to screening (p <0.01); $^{\#}$, significant trend to placebo (p <0.1); $^{\#\#}$, significant to placebo (p <0.05). Values are shown as means ± SDs.

3.5 Safety evaluation

There were no significant differences in examinations of physiology, hematology, and blood biochemistry between the screening and

end of the study (week 4) for either the low-dose, high-dose or placebo groups; fluctuations in values before and after the test were within physiological variations (**Tables 6-8**). In addition, no adverse events attributable to the test foods were observed in this study.

Table 6. Physical analysis

Parameter	Standard value	Group	Screening	Week 4
Weight (kg)	—	Placebo	59.6 ± 9.9	58.7 ± 10.1
		Low-dose	60.0 ± 10.1	59.3 ± 9.4
		High-dose	58.6 ± 10.3	57.5 ± 9.9
BMI (kg/m^2)	16.0-30.0	Placebo	22.4 ± 2.9	22.0 ± 3.1
		Low-dose	22.1 ± 3.1	21.9 ± 2.8
		High-dose	21.8 ± 2.5	21.4 ± 2.6
SBP (mmHg)	<150	Placebo	113.5 ± 12.8	111.5 ± 10.8
		Low-dose	112.7 ± 14.2	116.7 ± 13.8
		High-dose	115.1 ± 12.8	118.4 ± 11.0
DPB (mmHg)	<100	Placebo	75.9 ± 8.1	74.0 ± 7.0
		Low-dose	75.1 ± 11.1	77.1 ± 11.8
		High-dose	77.6 ± 8.2	78.7 ± 7.6
P (bpm)	50-100	Placebo	72.2 ± 9.2	72.6 ± 9.7
		Low-dose	72.0 ± 9.1	76.1 ± 10.5
		High-dose	72.8 ± 12.5	75.9 ± 12.0

Values are shown as means ± SD.

Table 7. Hematological analyses

Parameter	Standard value		Group	Screening	Week 4
	Male	Female			
WBC ($x10^3/\mu L$)	3.5-9.7		Placebo	6.04 ± 1.19	6.67 ± 1.40
			Low-dose	5.46 ± 1.08	6.54 ± 1.69
			High-dose	5.79 ± 1.45	6.26 ± 1.49
RWC ($x10^4/\mu L$)	438-577	376-516	Placebo	469 ± 35.9	464 ± 32.4
			Low-dose	463 ± 35.0	462 ± 43.8
			High-dose	467 ± 46.8	46.2 ± 51.8
Hb (g/dL)	13.6-18.3	11.2-15.2	Placebo	14.1 ± 1.2	13.9 ± 1.0
			Low-dose	14.1 ± 1.2	14.1 ± 1.3
			High-dose	14.2 ± 1.3	13.9 ± 1.4
HCT (%)	40.4-51.9	34.3-45.2	Placebo	44.0 + 3.5	43.0 ± 2.8
			Low-dose	44.4 ± 3.3	43.5 ± 3.5
			High-dose	44.2 ± 3.8	43.1 ± 3.8
PLT ($x10^4/\mu L$)	14.0-37.9		Placebo	29.6 ± 6.0	29.6 ± 5.9
			Low-dose	26.4 ± 4.3	26.9 ± 4.1
			High-dose	27.8 ± 4.8	27.1 ± 5.1

Values are shown as means ± SD.

4. Discussion

In this study, a 4-week, double-blind, parallel-group study was conducted to evaluate the effects of oral consumption of CJ peel powder[10] on allergy-like symptoms in healthy Japanese men and women with subjective symptoms of eye and nose discomfort in daily life.

In the comparison of responses to JRQLQ No. 1 (**Table 3**) between the high-dose group and placebo group, the high-dose group was significantly lower than the placebo group in the scores of "nasal and eye symptoms" at week 4, and "blocked nose" at weeks 2 and 4. In the comparison of the changes in scores (difference from the pre-observation period) on the Nasal and Eye Symptom Questionnaire, the decrease in "rhinorrhea" in the high-dose group was significantly greater than that in the placebo group in both the first and second half of the study period (**Table 5**). These results indicate that intake of high doses (1,000 mg/day) of CJ peel powder significantly reduced eye and nose discomfort, nasal congestion, and rhinorrhea.

In the comparison between the low-dose group and the placebo group regarding JRQLQ No. 1, the mean value of the low-dose group was lower than that of the placebo group for all endpoints although none of the differences were significant (**Table 3**). The changes in scores (difference from the pre-observation period) on the Nasal and Eye Symptom Questionnaire showed that the decrease in "paroxysmal sneezing" in the low-dose group was significantly greater than that in the placebo group in both the first and second half of the study period (**Table 5**). The decrease in "rhinorrhea" was significantly greater in the low-dose group than in the placebo group

in the first half of the study period and tended to be greater in the second half. Compared to the results of the high-dose group, the results for "paroxysmal sneezing" showed no dose-dependent effect, so an accidental effect cannot be ruled out, but the results for "rhinorrhea" showed a dose-dependent effect, indicating that the low-dose group also had a relieving effect on "rhinorrhea".

Table 8. Biochemical blood analyses

Parameter	Standard value		Group	Screening	Week 4
	Male	Female			
TP (g/dL)	6.5-8.2		Placebo	7.3 ± 0.3	7.3 ± 0.3
			Low-dose	7.2 ± 0.3	7.3 ± 0.3
			High-dose	7.2 ± 0.3	7.2 ± 0.4
TC (mg/dL)	150-219		Placebo	214 ± 36.7	205 ± 34.5
			Low-dose	209 ± 31.7	207 ± 32.1
			High-dose	204 ± 31.8	201 ± 33.8
LDL-C (mg/dL)	70-139		Placebo	122 ± 29.6	117 ± 30.9
			Low-dose	124 ± 22.7	125 ± 27.4
			High-dose	114 ± 27.0	114 ± 34.5
HDL-C (mg/dL)	40-80	40-90	Placebo	72.6 ± 14.5	68.5 ± 14.0
			Low-dose	67.5 ± 12.3	65.1 ± 11.4
			High-dose	71.1 ± 22.8	67.9 ± 24.5
TG (mg/dL)	50-149		Placebo	75.5 ± 41.0	75.7 ± 40.0
			Low-dose	84.0 ± 41.5	83.0 ± 37.3
			High-dose	79.0 ± 40.3	85.1 ± 51.6
BUN (mg/dL)	8.0-20.0		Placebo	13.1 ± 3.5	13.1 ± 4.9
			Low-dose	13.0 ± 2.2	14.1 ± 3.6
			High-dose	12.6 ± 4.5	13.3 ± 3.6
CRE (mg/dL)	0.65-1.09	0.46-0.82	Placebo	0.7 ± 0.1	0.7 ± 0.1
			Low-dose	0.7 ± 0.2	0.8 ± 0.2
			High-dose	0.7 ± 0.2	0.7 ± 0.2
UA (mg/dL)	3.6-7.0	2.7-7.0	Placebo	5.0 ± 1.3	5.0 ± 1.3
			Low-dose	5.2 ± 1.2	5.3 ± 1.2
			High-dose	5.3 ± 1.2	5.6 ± 1.2
T-Bil (mg/dL)	0.3-1.2		Placebo	0.7 ± 0.2	0.8 ± 0.2
			Low-dose	0.7 ± 0.3	0.8 ± 0.4
			High-dose	0.8 ± 0.3	0.9 ± 0.4
AST (U/L)	10-40		Placebo	22.8 ± 6.2	21.4 ± 5.5
			Low-dose	22.3 ± 6.1	22.3 ± 8.6
			High-dose	21.8 ± 5.8	23.1 ± 12.0
ALT (U/L)	5-45		Placebo	22.2 ±14.2	20.0 ± 11.3
			Low-dose	20.9 ± 10.8	20.2 ± 11.9
			High-dose	19.7 ± 11.1	20.2 ± 15.4
ALP (U/L)	38-113		Placebo	65.7 ± 20.4	66.7 ± 18.9
			Low-dose	57.6 ± 14.3	62.1 ± 15.7
			High-dose	57.9 ± 17.9	60.4 ± 17.5
LDH (U/L)	120-245		Placebo	174 ± 30.3	171 ± 27.8
			Low-dose	168 ± 22.6	173 ± 29.6
			High-dose	166 ± 27.8	167 ± 25.3
γ-GPT (U/L)	<79	<48	Placebo	24.5 ± 14.8	24.1 ± 20.7
			Low-dose	26.2 ± 11.6	24.8 ± 10.6
			High-dose	25.2 ± 15.9	23.3 ± 12.6
CPK (U/L)	50-230	50-210	Placebo	118 ± 87.7	114 ± 78.9
			Low-dose	112 ± 41.9	125 ± 66.8
			High-dose	110 ± 80.4	100 ± 51.4
GLU(mg/dL)	70-109		Placebo	91.8 ± 7.6	88.8 ± 5.3
			Low-dose	91.3 ± 8.3	90.9 ± 9.4
			High-dose	89.7 ± 7.5	94.5 ± 21.3

Values are shown as means ± SDs.

In this study, there was an improvement in symptoms in the high-dose, low-dose, and placebo groups compared to the pre-observation period, which may be attributed to decrease in pollen levels during the study period compared to the time of pre-observation period[12]. In addition,

the period of this study coincided with the time when people were required to refrain from going out and to wear masks when going out and in the office as countermeasures against COVID-19, and these factors may have impacted the allergen exposure among the study participants. These circumstances suggest that during this study, it was more difficult than usual to find a significant difference from the placebo group. Furthermore, the declaration of a state of emergency due to the expansion of COVID-19 during the study may have disrupted the lives of some participants, resulting in more cases of exclusion.

In the safety evaluation of the test food, there were no significant differences in examination of physiology (**Table 6**), hematology (**Table 7**), and blood chemistry (**Table 8**) among the low-dose, high-dose, and placebo groups, and the pre- and post-test variations were within physiological levels. In addition, there were no adverse events caused by the test foods in this study. These results indicate that the CJ fruit peel food in this study is safe at both low and high doses.

Comparing the results of this study with those of previous reports, the anti-inflammatory and anti-allergic effects of CJ fruits and narirutin, which is abundantly contained in the fruit, have been recognized in cell[6,7,9] and animal[3,8] experiments, as well as in oral intake intervention studies using before-and-after comparison[6,7] tests. In addition, the CJ peel powder[10] used in this study has been shown to be safe in non-clinical studies, healthy volunteers, and patients with atopic dermatitis (AD), and to be useful for patients with AD[2]. The results of this study are consistent with the anti-allergic effects reported above. To our knowledge, this is the first double-blind, parallel-group study showing the effects of oral consumption of any part of CJ fruit.

5. Conclusion

A randomized, parallel-group human food study of CJ peel powder[10] using a high-dose group (1,000 mg/day), a low-dose group (500 mg/day), and a placebo group showed that high-dose CJ peel powder improved the “eye and nasal symptoms” of allergic rhinitis symptoms, while low-dose CJ peel powder improved “nasal symptoms”. In addition, the safety of 4-week continuous intake of CJ peel powder was demonstrated.

Ethics statement

The Clinical Trial Review Committee of Hakusui-Kai Suda Clinic Medical Corporation approved this study (Approval number: 2021-004). All study participants provided informed consent. The study protocol for this study was registered in advance with the University Hospital Medical Information Network (UMIN) (UMIN000043224).

Conflict of interest

This study was funded by Jabara Laboratory Co., Ltd., but the study was conducted by a third-party organization. Yoshinobu Murakami belongs to the Department of Aesthetics and Health Sciences, Wakayama Medical University, which is funded by Jabara Laboratory Co., Ltd. Kiyoshi Nakamura was involved in this study as the investigator, but no research expenses or honoraria were paid to him by Jabara Laboratory Co., Ltd.

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CASE REPORT

Acute colonic pseudo-obstruction following nivolumab and ipilimumab combination therapy for metastatic melanoma

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ABSTRACT

Immune-related adverse events (irAEs) are commonly observed in patients treated with immune checkpoint inhibitors (ICI), and prompt diagnosis and treatment of irAEs is of utmost importance. Gastrointestinal (GI) events are among the most frequent irAEs and the hallmark symptom is diarrhea. Intestinal hypomotility as irAEs is exceedingly rare, and needs wider recognition given that the presentation is insidious.

Here, we report a case of 79-year-old woman with metastatic melanoma under nivolumab and ipilimumab combination therapy. She developed ileus symptom, and was diagnosed with acute colonic pseudo-obstruction. The symptom relieved soon after administering high-dose prednisolone five days after the onset. ICI therapy was discontinued.

Intestinal hypomotility as GI irAEs is exceedingly rare and there have been five reported cases to our knowledge. In reviewing past cases, we speculate that the prompt initiation of corticosteroids resulted in a favorable outcome. Our case illustrates that early recognition of these rare irAEs is essential in order to ensure prompt treatment.

Keywords: Immune-related Adverse Events; Immune Checkpoint Inhibitor; Melanoma; Acute Colonic Pseudo-obstruction; Ileus

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1. Introduction

Immune checkpoint inhibitors (ICI) targeting cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) and programmed death-1/ligand (PD-1/PD-L1) have become a new standard of treatment in several cancers including melanoma[1]. Immune-related adverse events (irAEs) of CTLA-4 and/or PD-1/PD-L1 inhibition are commonly observed in the skin, gastrointestinal tract, liver and endocrine systems and include pruritus, rash, nausea, diarrhea and thyroid disorders[2-4]. In contrast to the direct cytotoxic action of traditional antineoplastic agents, ICI enhance antitumour T-cell activity. This leads to a systemic loss of tolerance, with resulting irAEs, of which gastrointestinal (GI) irAEs are among the most frequent and severe, and the hallmark symptom is diarrhea[5]. Here, we report an atypical and insidious presentation of GI irAEs during ICI therapy, and speculate early recognition and prompt treatment of this rare adverse effect lead to favorable outcome, reviewing past cases.

2. Case report

A 79-year-old female with metastatic amelanotic vulvar melanoma after three doses of nivolumab and ipilimumab as second-line treatment was admitted to our hospital. She was diagnosed with right vulvar melanoma with sentinel lymph metastasis, and underwent palliative resection of the primary tumor seven months ago. After the surgery, she began nivolumab infusion every two weeks. Liver metastasis was detected at the ninth nivolumab infusion and her treatment was switched to combination therapy with nivolumab and ipilimumab every three weeks. At the time of admission, she complained anorexia and fatigue, which had begun one day after the last administration of nivolumab and ipilimumab. She did not present diarrhea, nausea or abdominal pain, and had no history of abdominal surgery. She underwent palliative resection of the primary tumor seven months before and began nivolumab treatment. Liver metastasis was detected at the ninth nivolumab infusion and her treatment was switched to combination therapy with nivolumab and ipilimumab. After the first infusion of the combination treatment, she was diagnosed with adrenal insufficiency due to immunotherapy-related hypophysitis and started on corticosteroid replacement treatment.

After admission, stress-dose corticosteroid therapy was started. Since day 4 of admission, she developed intermittent fever. Laboratory data showed white blood cell count 5,840/μL with 75.9% neutrophils; AST 68U/L; ALT 37U/L; LDH 428U/l; ALP; 281U/L; CRP 5.8 mg/dL; and normal serum amylase, and creatinine levels. Two sets of blood culture and cytomegalovirus antigen were negative. On day 5 of admission, she presented abdominal distention, vomiting and constipation. Clinical examination revealed moderate abdominal distension painless to palpation with decreased bowel sounds, unaccompanied by signs of peritoneal irritation or low back pain. Abdominal CT scan revealed distended large intestine without discernible transition point or tumor lesions (**Figure 1a**). Patient received laxatives without success. On day 6 of admission, abdominal X-ray showed niveau formation in the entire large intestine (**Figure 1b**). Colonoscopy revealed mild colitis affecting continuously from the rectum to the descending colon (**Figure 2**). Sigmoid colon biopsies showed cryptitis infiltrated with neutrophils, lymphocytes and plasma cells without granuloma or intranuclear inclusion bodies (**Figure 3**).

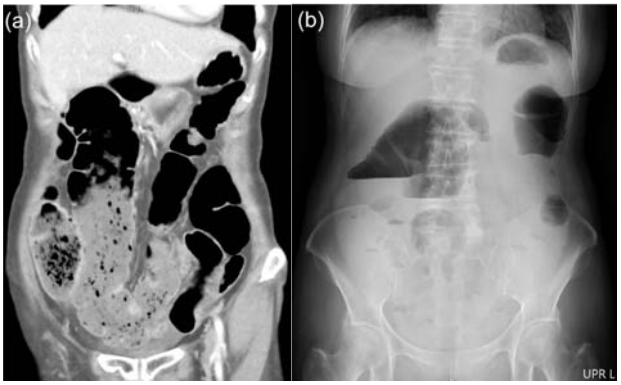

Figure 1. Radiological findings**. (a)** On day 5 of admission, the coronal plane of abdominal CT scan revealed a highly distended large bowel loop. **(b)** Abdominal X-ray on day 6 of admission revealed niveau formation in the entire large intestine.

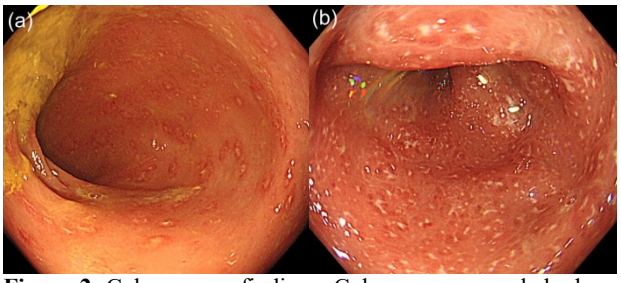

Figure 2. Colonoscopy findings**.** Colonoscopy revealed edematous mucosa, erythema, loss of vascular markings and mucosal friability continuously in the rectum **(a)** and sigmoid colon **(b)**.

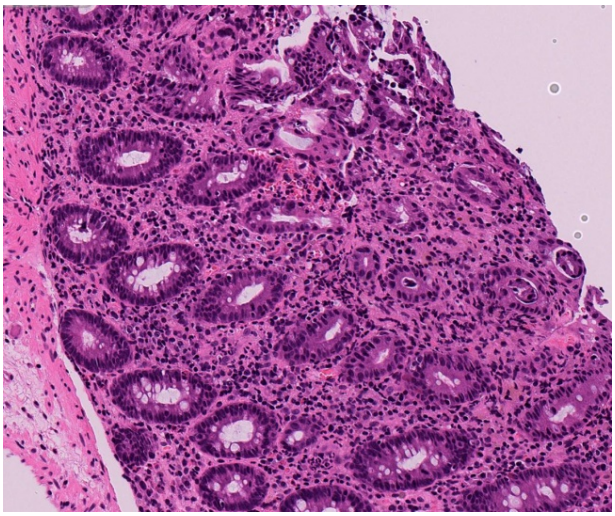

Figure 3. Sigmoid colon biopsy findings**.** Sigmoid colon biopsies showed cryptitis and crypt abscess infiltrated with neutrophils, lymphocytes and plasma cells without granuloma or intranuclear inclusion bodies.

No pathogenic bacteria were detected in the biopsy culture. Metastatic tumors to the spinal

cord and brain were not detected and paralytic ileus due to metastasis to the brain or spinal cord was deniable. She was not administered antimuscarinics or opioids which can cause drug-induced paralytic ileus. After excluding the possibility of infection, malignancy and drug-induced paralytic ileus, we diagnosed the patient with acute colonic pseudo-obstruction related to ICI. ICI treatment was ceased, and intravenous prednisolone (1 mg/kg/day) started on day 10 of admission. On day 20, she defecated normally. After switching to oral administration, prednisolone was tapered and discontinued after 8 weeks. Abdominal symptoms did not recur, and no abnormal findings were detected in colonoscopy after three months in spite of the progressive disease.

3. Discussion

Our report describes a rare presentation of acute colonic pseudo-obstruction during ICI therapy. The abdominal CT scan revealed no discernible obstructive point, and paralytic ileus due to metastasis to the brain or spinal cord was deniable. Infectious or drug-induced paralytic ileus was also deniable. We concluded that acute colonic pseudo-obstruction was induced by ICI therapy, and administered high-dose prednisolone five days after the onset. ICI therapy was discontinued. The symptom relieved within ten days, and did not recur regardless of progressive disease of melanoma. According to the Naranjo Algorithm, the symptom described in the patient case yielded an adverse drug reaction probability score of 7, which indicates a probable adverse event[6].

Intestinal hypomotility as GI irAEs is exceedingly rare and there have been five reported cases to our knowledge[7-11]. These cases paint a highly variable picture; in three cases, intestinal hypomotility developed late after initiating ICI treatment (11-cycle pembrolizumab, 14-cycle nivolumab and 8-cycle pembrolizumab, respectively)[7-9]. The other two cases, reported from the same institution, developed GI symptoms acutely (2 cycles of ipilimumab and 1 cycle of combination therapy with nivolumab plus ipilimumab, respectively) and were examined by autopsy[10,11]. The former died of unrelated cause, and the latter was refractory to high dose steroids and fatal[10,11]. In both cases, they identified myenteric ganglionitis at autopsy[10,11]. In the former case, autopsy slides revealed lymphocytes infiltrating myenteric ganglia throughout the gastrointestinal tract[10]. The latter case showed near complete loss of ganglion cells within the myenteric and submucosal plexuses, and no conspicuous inflammatory infiltrate was seen around the ganglia at the time of autopsy, suggesting a 'burned out' phase of illness[11].

Overlooking the past four cases of irAE-related intestinal hypomotility excluding the one case which resulted in unrelated death, corticosteroids seem to be the treatment of choice, resulting in complete resolution in one case, limited efficacy in another case, no response and/or death in two cases[7-9,11]. Among the five cases, two reports mentioned the timing of initiation of corticosteroid administration. In one case, high-dose prednisone administration started seven days after the onset, and resulted in gradual improvement of symptoms and immunotherapy was restarted[8]. The other case was started on high-dose prednisone treatment on the sixteenth day of onset, but refractory and fatal[11]. Prompt initiation of therapy appears to be necessary to attain favorable response[8,11].

Our case presented with acute colonic pseudo-obstruction, which relieved soon after administering high-dose prednisolone five days after the onset. Although we could not obtain deep biopsy, we consider myenteric ganglionitis related with immunotherapy contributed to pseudo-obstruction since the severity of colitis examined by colonoscopy was relatively mild. We speculate that the prompt initiation of corticosteroids resulted in a favorable outcome. Our case illustrates that early recognition of these rare irAEs is essential in order to ensure prompt treatment.

Consent

Written informed consent was obtained for publication of this case report and any accompanying images.

Declaration of conflicting interests

The authors declare that there is no conflict of

interest with respect to the research, authorship, and/or publication of this article.

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CASE REPORT

Allogeneic bone marrow transplantation possibly induces a localized type of porokeratosis

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ABSTRACT

A 15-year-old girl underwent allogenic bone marrow transplantation for neuroblastoma. A few years later, she noticed a round lesion on her left buttock. Since the lesion had been asymptomatic and never grown, more than 20 years had passed before she saw a local doctor to consult about it. Although the lesion was suspected to be tinea corporis, no fungi were found on microscopic examination. Subsequently, administered topical corticosteroids were not effective. She was referred to our hospital for further evaluation, and a skin biopsy confirmed the diagnosis of porokeratosis. There was a possibility that chemotherapy, total body radiation, or immunosuppressive therapy associated with allogeneic bone marrow transplantation was involved in the development of porokeratosis. Numerous cases of acquired porokeratosis in immunocompromised status have been observed; as for those after allogenic bone marrow transplantation, 12 cases have been reported in the English literature, 4 of which had only one or a few lesions on a limited area of body surface. Our case was relatively uncommon in that the lesion was solitary and comparatively large. In a localized type of porokeratosis, it was suggested that a malignant skin tumor developed earlier than in other types. Careful follow-up for malignant transformation is especially required.

Keywords: Porokeratosis; Localized Type; Bone Marrow Transplantation; Immunosuppression; Malignant Transformation

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1. Introduction

Porokeratosis is a rare hyperkeratotic disorder arising from abnormal clones of keratinocytes. The risk factors for its genesis include inheritance, trauma, and ultraviolet light[1]. Since MacMillan reported a case of porokeratosis after renal transplantation in 1974[2], immunosuppression has also been believed to be a risk factor. Immunosuppression-associated porokeratosis is more often characterized by multiple lesions rather than a single lesion[3]. We herein report a case of localized porokeratosis after allogeneic bone marrow transplantation for neuroblastoma.

2. Case presentation

A 42-year-old woman presented with an asymptomatic round lesion on her left buttock. She consulted a local doctor, and the lesion was suspected to be tinea corporis, but no fungi were found by microscopy. Although topical corticosteroids were prescribed, the lesion did not improve, and she was referred to our hospital for further evaluation. She had a solitary, 6.5 × 3.5 cm, annular erythematous patch with a brownish hyperkeratotic border (**Figure 1**). She noticed it at the

age of 17 or 18. Since then, its size had not changed for more than 20 years. No rash was found on other areas. She denied any family history. A biopsy of the brownish border of the lesion showed a cornoid lamella, which was a column of tightly fitted parakeratotic cells in the upper epidermis (**Figure 2**). She was diagnosed with porokeratosis. The lesion has been followed up clinically without any specific therapy.

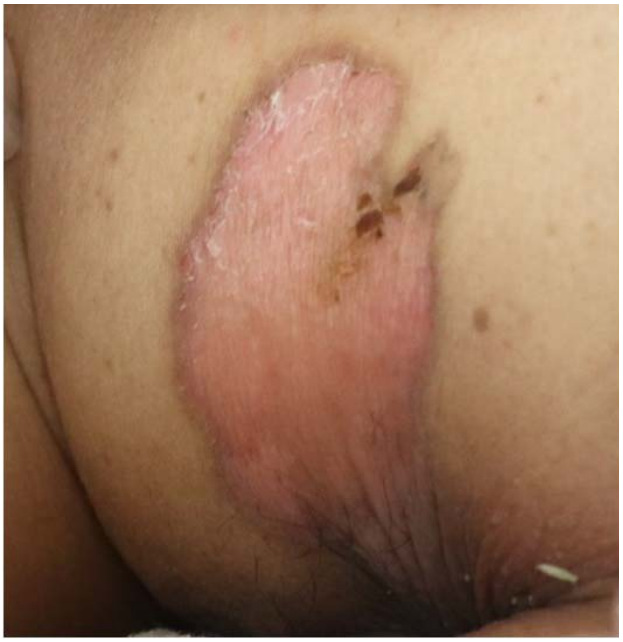

Figure 1. An annular erythematous patch with a brownish hyperkeratotic border on the left buttock**.**

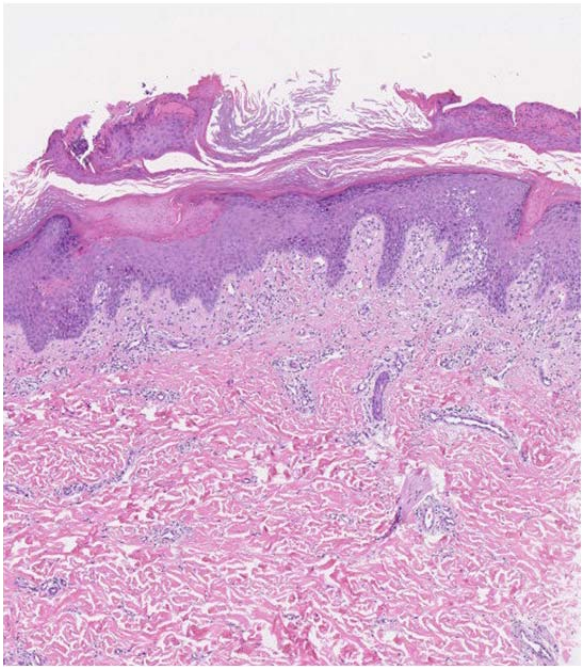

Figure 2. A column of tightly fitted parakeratotic cells in the upper epidermis, which is consistent with a cornoid lamella (hematoxylin and eosin ×10)**.**

According to her medical record obtained in our hospital, she was diagnosed with neuroblastoma at the age of 14 by close examination of abdominal pain. Surgical operation and adjunctive chemotherapy were performed the following year. In addition, she underwent allogeneic bone marrow transplantation, with high-dose chemotherapy and total body radiation as preparative therapy. Immediately after allogeneic bone marrow transplantation, the immunosuppressive agent cyclosporine was initiated. Two months later, chronic graft-versus-host disease developed, for which treatment with prednisolone 45 mg daily was started. A good clinical response was obtained, and prednisolone was tapered over 5 months.

3. Discussion

Porokeratosis was first described by Mibelli in 1893[4]. It is a disorder of epidermal keratinization and characterized by a distinct peripheral keratotic ridge that corresponds histologically to the cornoid lamella. Although no unified classification standard for it is yet to be created, it has been classified in Japan, on the basis of its clinical characteristics as follows: classical porokeratosis, localized porokeratosis, linear porokeratosis, disseminated superficial porokeratosis, and disseminated superficial actinic porokeratosis[5]. In our case, a single lesion of 6.5 × 3.5 cm was observed only on the left buttock, and it was therefore considered to be localized porokeratosis.

Porokeratosis has been observed in various types of immunosuppression, including that after solid organ transplantation[2,6], systemic corticosteroids[7], electron beam radiation[8], and biological agents[9]. The development of porokeratosis during the course of human immunodeficiency virus infection has also been reported[10]. That is to say, genetic predisposition and a variety of triggers such as immunosuppressive drugs may be associated with the occurrence of porokeratosis. Recently, causal mutations of several genes (MVK, PMVK, MVD, and FDPS) have been identified[11,12], and it has been shown that approximately one per 400 Japanese individuals is estimated to have a pathogenic mutation in MVD[13]. Each skin lesion of disseminated superficial actinic porokeratosis origi-

nates from a postnatal keratinocyte clone with a different second-hit genetic event in the wild type allele of the corresponding gene, and linear porokeratosis derives from a single prenatal clone of keratinocytes with a second-hit genetic event[13].

Although the exact timing of porokeratosis onset in our case is unknown, the patient noticed the lesion on her left buttock within a few years after allogeneic bone marrow transplantation. There is a possibility that chemotherapy or total body radiation before bone marrow transplantation, or immunosuppressive therapy after bone marrow transplantation led to porokeratosis, but the identification of the causative one is difficult because the combined effects of these therapies may have been involved. We speculate that the patient exhibits monoallelic germline mutations in genes encoding mevalonate pathway enzymes such as MVD, and total body radiation triggers an individual second hit genetic change in the wild-type allele of the corresponding gene specifically in the epidermis. In addition, loss of immunosurveillance caused by chemotherapy or immunosuppressive therapy may facilitate the proliferation of abnormal keratinocyte clones. However, further studies are required to elucidate that localized porokeratosis is caused by a similar mechanism to that of disseminated superficial actinic porokeratosis. Alexis and colleagues argued that, in the case of bone marrow transplantation, the frequent lack of concurrent immunosuppressive therapy at the time of diagnosis of porokeratosis suggests a more complex association with immunosuppression than that after solid organ transplantation[3].

We summarized cases of porokeratosis after allogeneic bone marrow transplantation that have been reported in the English literature (**Table 1**)[3,6,14-18]. In 4 of 12 cases, only one or a few lesions were present on a limited area of body surface as in our case[3,6]. The lesion size of our case was much larger than any reported lesion sizes shown in **Table 1**.

Table 1. Cases of porokeratosis after allogenic bone marrow transplantation reported in the English literature

Age/Sex	Anatomic location	Number of lesions	Size (cm)	Post-transplantation time (years)	Reported year [reference]
19/M	Leg, buttock	A small number	0.5–1.0	1	1985[14]
38/M	Arm	1	Not stated	Not stated	1992[6]
32/M	Thigh, buttock, flank	Not stated	Not stated	Not stated	1995[15]
37/M	Flank, thigh, buttock	3	1.0, n/a for thigh and buttock	3, 4	2006[3]
62/M	Leg	1	1.5	6	2006[3]
58/M	Preauricular region, antihelix, thigh, calf	5	0.4, 0.5, n/a for thigh and calf	3, 4, 5	2006[3]
42/F	Popliteal fossa	2	0.8, 1.0	13	2006[3]
42/M	Thigh	1	0.5	1	2006[3]
38/M	Both extremities, trunk	Multiple	0.5–2.5	3	2010[16]
13/M	Leg, arm, abdomen, face	Multiple	0.5–1.5	1	2012[17]
1/M	Thigh, axilla	2	Not stated	Not stated	2015[18]
12/M	Popliteal fossa, cervical region	5	Not stated	Not stated	2015[18]

It has been reported that administration of cyclosporine led to malignant disorders[19,20]. Some immunosuppressive agents used after bone marrow transplantation may be associated with cancer progression. A possible complication of porokeratosis is Bowen's disease, squamous cell carcinoma or basal cell carcinoma. A study showed that a localized type of porokeratosis developed a malignant skin tumor in an average of 22.3 years, which was significantly earlier than other types[5]. The lesion in our case is relatively large, and we carefully follow-up the patient so as not to overlook malignant transformation.

Conflict of interest

The authors declare no potential conflicts of interest.

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CASE REPORT

A case of lichen planopilaris associated with lichen planus following Blaschko lines successfully treated with topical corticosteroid

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ABSTRACT

Lichen planopilaris (LPP) is characterized by erythematous, keratotic follicular papules and cicatricial alopecia. LPP, the most common cause of cicatricial alopecia, is usually seen in women and causes significant psychosocial morbidity. We describe here a 42-year-old woman with a 6-month history of hair loss accompanied by itching on the scalp. Dermatological examination revealed patchy cicatricial alopecia in the vertex and band-like purple flat patches and plaques following the Blaschko lines on the right half of the body, together with post-inflammatory hyperpigmentation. Histopathological examination of the scalp biopsy was consistent with LPP, while thigh biopsy was consistent with lichen planus. With the histopathological and clinical evidence, our patient was evaluated as LPP associated with LP and successfully treated with topical corticosteroid. A few LPP and LP cases following the Blaschko lines have previously been reported separately. However, LPP, together with LP following Blaschko lines, have not been reported in the same patient.

Keywords: Lichen Planus; Lichen Planopilaris; Blaschko Lines

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1. Introduction

Lichen planopilaris (LPP) is distinguished by erythematous, keratotic follicular papules and cicatricial alopecia, localized especially on the vertex. LPP, the most common cause of cicatricial alopecia, is seen mostly in women between the ages of 40 and 60 and constitutes significant psychosocial morbidity. LPP may be accompanied by skin and mucosal symptoms of lichen planus (LP). Frontal fibrosing alopecia and Graham-Little-Picardi-Lassueur syndrome (GLPLS) are considered as variants of LPP[1]. The exact aetiology is not fully understood but is thought to be associated with an inflammatory response mediated by T lymphocytes targeting follicular antigens[2].

In a few cases, both LPP and LP cases following Blaschko lines have been reported separately in the literature. However, this is the first case report, to our knowledge, of LPP associated with LP following Blaschko lines to be reported in the English literature.

Lichen planus is a disease in which corticosteroids and various immunosuppressive treatments are used in the treatment. In our case, we applied intralesional corticosteroid treatment to the scalp and potent topical corticosteroid for skin lesions.

2. Case report

A 42-year-old female patient was admitted to our clinic with a 6-month history of hair loss accompanied by itching on the scalp. She developed rashes on her body 4 years before admission. Initially, itchy band-like rashes on the right thigh, inguinal and pubic areas were pink-red but eventually became dark red and purple. She had used topical corticosteroids for only lesions on the thigh, inguinal and pubic areas for varying durations in the first years of the disease and 3 months before her admission to our clinic with a partial response. Her past and family history was unremarkable except for hypothyroidism. She did not have a history of herpes zoster before her symptoms appeared. Dermatological examination revealed patchy cicatricial alopecia areas in the vertex (**Figure 1**).

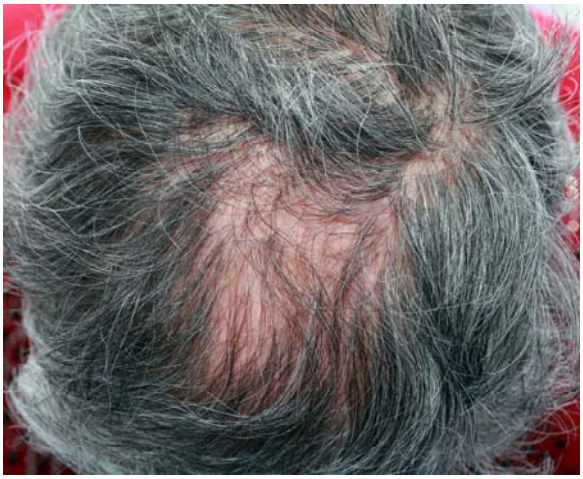

Figure 1. Lichen planopilaris; Patchy cicatricial alopecia areas in the vertex**.**

There were band-like patches and plaques following the Blaschko lines on the right half of the body. Purple flat papules and plaques, together with post-inflammatory hyperpigmentation, were observed in the right inguinal region, pubis, and thigh (**Figure 2**). There were no alopecic areas in the axillary and pubic regions. Scalp dermoscopy showed peripilar casts and cicatricial alopecia patches on the erythematous base where hair follicle openings could not be selected. Examination of mucosal surfaces and nails were normal. Routine laboratory examinations, including viral hepatitis markers, were also normal ranges. Histopathological examination of the scalp biopsy revealed lichenoid infiltration in the basal layer of the follicular epithelium and perifollicular lymphocyte infiltration, consistent with LPP. Thigh biopsy was consistent with lichen planus with hyperkeratosis, regional hyper-granulosis, irregular acanthosis, inflammatory cell infiltration in the upper dermis, and pigment incontinence.

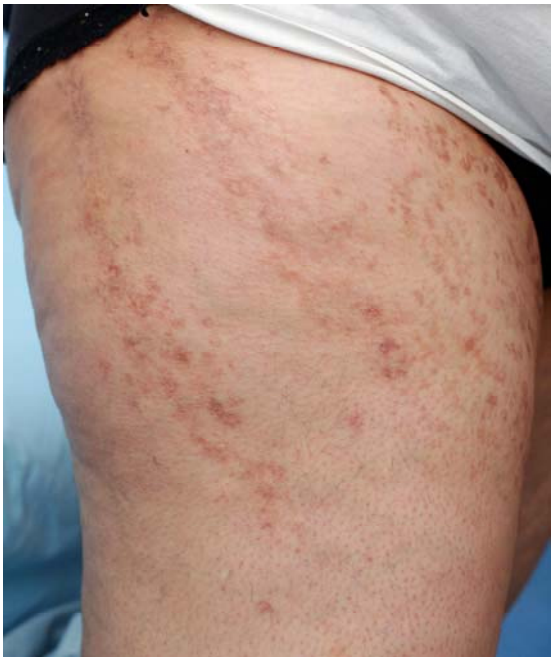

Figure 2. Linear lichen planus; Band-like patches and plaques together with post-inflammatory hyperpigmentation following the Blaschko lines on the right thigh**.**

3. Discussion

LP is an immune-mediated mucocutaneous disease with a broad clinical spectrum. The association of LP and LPP has been reported only in individual patients[2]. There is one exception to this. Vulvovaginal-gingival lichen planus (VVG-LP) is a unique form of LP comprising a triad of symptoms: vulval, vaginal and gingival LP lesions. Recently, Olszewska *et al.*[3] reported that VVG-LP shows an increased association with LPP. They noted that 75% of 16 patients with VVG-LP had LPP of the scalp. Our patient had linear LP, and she did not have mucosal involvement. Linear LP has been described as a zosteriform distribution in the healed herpes zoster regions, following Blaschko lines. It may also develop as an isotopic response in areas previously exposed to trauma (koebnerization). However, we did not detect any history of herpes

zoster or trauma leading to koebnerization. We define here a patient with concomitant LPP and linear LP. In our case, LPP appeared after LP started. To the best of our knowledge, LP preceding the onset of LPP has not been previously reported.

LPP and LP share a common pathogenic pathway, including autoimmune response against some common antigens and/or overexpression IL-23/IL-17 axis[4]. In our case, LPP developed 4 years after the appearance of LP. Some authors consider LPP as the follicular variant of LP[2]. Therefore, it can be speculated that LPP may be developed by following the LP with the immunogenic co-pathway activation. In our case, in contrast to LPP, LP has a pronounced segmental involvement that was following the Blaschko lines. This association may also be explained by type 2 segmental mosaicism, which results in loss of the corresponding wild-type allele occurring at a very early developmental stage in a heterozygous embryo. When this hypothesis is accepted, our case confirms that LPP and LP are only different spectra of the same disease

In conclusion, LPP accompanying linearly located LP in one half of the body has not been reported in the English literature. This situation can be a coincidence. LPP may be developed by following the LP with the immunogenic co-pathway activation or reflect another example of type 2 segmental mosaicism.

Conflict of interest

The authors declare that they have no conflict of interest.

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MINI-REVIEW

Inflammaging in skin and intrinsic underlying factors

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ABSTRACT

Aging of organs starts from the time of birth and continues throughout life. Aging of skin can be divided into two distinct types — intrinsic aging and extrinsic, based on the fact that the skin is the outermost organ exposed to the external environment. However, despite their different histological features and triggers, intrinsic and extrinsic aging share common biochemical mechanisms. β-galactosidase, $p16^{INK4a}$, and senescence-associated secretory phenotype (SASP) factors are detected in skin cells as biomarkers of senescence. In particular, inflammatory cytokines, the constituents of SASP, play pivotal roles in "inflammaging" which is a concept involving the relationship between aging and low-grade inflammation. In this review, the features of skin aging and its underlying mechanism of skin aging are summarized.
Keywords: SASP; Inflammaging; Skin

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1. Introduction

Aging of organs starts from the time when one is born and continues throughout life. Thus, the strategies to prevent chronological tissue dysfunction have become an important issue in increasing elderly societies. Since Hayflick and Moorhead reported the finite proliferative capacity of cultured normal human fibroblasts[1], researchers have contended the involvement of cell senescence in organ aging. However, accumulating evidence has established obvious roles of senescent cells, which are defined by irreversible cell-cycle arrest, resistance to apoptosis, and senescence-associated secretory phenotype (SASP), in physiological and pathological states[2-4].

In the skin, aging can be divided into two distinct types: intrinsic aging and extrinsic aging. Intrinsic aging is an inevitable physiological process and characterized by dry skin, fine wrinkles, and gradual dermal atrophy. On the other hand, extrinsic aging is induced by environmental factors, including air pollution and sun exposure, and is characterized by coarse wrinkles, loss of elasticity, and rough texture appearance[5,6]. Similar to other organs, senescent cells accumulate in intrinsically and extrinsically aged skin and contribute to skin aging. In this review, the skin environment of senescent cells is summarized.

2. Intrinsic skin aging

Intrinsic skin aging is a process of physiological change involving photo-protected areas. Intrinsically aged skin is clinically characterized by dryness, fine wrinkles, and a histologically thinner epidermis and flattened dermal-epidermal junction[7,8]. Clinical features are induced by a significant reduction in surface lipid production with

chronological aging, whereas stratum corneum hydration and transepidermal water loss are modestly lowered or unchanged[9,10]. Thinning of the epidermis is induced by reduction of basal keratinocyte proliferation dependent on reduction of the nutrient flux through age-related flattened dermal-epidermal junctions, in which the area of the available exchange surface is reduced[11,12]. In the dermis, there are fewer fibroblasts in aged skin than in young skin[13]. In addition, the production of type I procollagen in intrinsically aged human skin is reduced, depending on the downregulation of TGF-β/Smad signaling[14]. In addition to aging-induced structural changes, presumably the cutaneous immunity becomes defective with age. A variety of bacterial, fungal, and viral infections markedly increase with age[15-17]. Toll-like receptors (TLRs), which are crucial pathogen pattern recognition receptors, are expressed in keratinocytes, fibroblasts, and skin-resident immune cells. Once triggered by ligands, TLRs lead to the production of inflammatory cytokines and initiation of immune responses[18,19]. Shaw *et al.* reported that the expression and function of TLRs diminished with age[20]. Furthermore, TLR ligand-induced production of inflammatory cytokines is reduced in circulating dendritic cells in older individuals[21]. This indicates that pathogen pattern recognition, which is the primary process of the innate immune system, is attenuated with age. Dendritic cells (DCs), which are the sentinels of the immune system, bridge the innate and adaptive immune system by sequestration and presentation of antigens to T cells. Diverse populations of DCs, including dermal DCs, dermal macrophages in the dermis, and Langerhans cells (LCs) represent DCs in epidermis[22,23]. A previous study reported that the absolute number of DCs and their $CD34^+$ precursors declined with age[24,25]. In addition to the reduction in the number of LCs in aged-skin, the migratory ability of LCs in aged skin is impaired because of attenuation of the responses to cytokine gradients, and the subsequent accumulation of LCs in regional lymph nodes is reduced[26-28]. In the whole skin of an average person, there are approximately 2×10^{10} T cells, including resident memory T cells (T_{RM}) and circulating memory T cells[29]. The $CD4^+$/$CD103^-$ T_{RM} cells are located in the dermis, whereas $CD4^+$/$CD103^+$ and $CD8^+$/$CD103^+$ T_{RM} cells are enriched in epidermis. Both $CD4^+$/$CD103^+$ and $CD8^+$/$CD103^+$ T_{RM} cells have more potent effector functions than circulating T cells, but have less proliferative capacity than that of the $CD103^-$ T_{RM} cells[30]. To investigate antigen-specific T cell responses, delayed-type hypersensitivity reactions (DTHs) represent the most informative *in vivo* experimental models. Previous studies have demonstrated that DTHs are impaired in older humans and mice[18,31-33]. The proportion of memory phenotype T cells increases with age and becomes predominant after midlife, whereas the total number of T cells is maintained throughout life[34]. Repeated antigen exposure during the lifespan induces exhausted T cells characterized by telomere shortening and expression of exhaustion markers such as PD1 and LAG3[35]. Moreover, continuous homeostatic proliferation induces dysfunctional $CD4^+$ T cells, named senescence-associated T cells, which are characterized by the expression of PD1 and LAG3, and abundant secretion of inflammatory cytokines. The proportion of senescence-associated T cells progressively increases with age[36,37]. Therefore, it is supposed that age-related dysfunction, including antigen recognition and presentation, and senescence in T cells reflects cutaneous immunity.

3. Extrinsic skin aging

Extrinsic aging is caused by several exogenous factors such as tobacco smoke, air pollution and ultraviolet (UV) rays. Out of these factors, UV affects aging the most; therefor, extrinsic aging is referred to as photoaging. UV is classified as UVA, UVB and UVC, depending on the wavelength. UVA (320–400 nm) and UVB (280–320 nm) reach the surface of the earth, while UVC (100–280 nm) is absorbed by the ozone layer. Although UVB has higher energy, UVB is mostly absorbed by the epidermis, owing to its shorter wavelength, while UVA, which has a lower energy, can penetrate into the dermis. Therefore, UVB is responsible for acute sunburn reactions in the epidermis, and UVA is considered as a major factor in chronic dermal photoaging. UV-irradiated epidermis thickens, in contrast to the thinner epidermis, is observed in intrinsically aged skin[38]. Tissue renewal in the epi-

dermis is dependent on proliferative cells in the basal layer, which include keratinocyte stem cells (KSCs) and transit amplifying (TA) cells[39]. Although the expression of integrin β1, which is a KSC marker, is reduced and the ratio between involucrin, a differentiation marker of keratinocytes, and integrin β1 is increased, aberrant suprabasal integrin β1 expression and enhanced expression of Ki-67, expressed in proliferating cells, are detected in chronic sun-exposed skin of the elderly. In addition, flow cytometric analysis revealed that integrin $\alpha6^{bri}$ $CD71^{bri}$ cell numbers are greater in sun-exposed epidermis than in sun-protected epidermis, suggesting that the proliferation of TA cells is increased in sun-exposed epidermis[40,41]. These results suggest that UV exposure induces a hyperproliferative state of epidermis in photoaged skin. Another clinical characteristic is the presence of coarse wrinkles in photoaged skin. Studies have demonstrated that the reduction of collagen type I formation in photodamaged human skin, depending on UV irradiation-induced matrix metalloproteinase (MMP) expression and synthesis inhibition by damaged collagen, contributes to UV irradiation-induced wrinkle formation[42-44]. Similarly, the suppression of collagen type IV, a component of the basement membrane, and collagen type VII, an anchoring fibril connecting fibroblasts to the basement membrane, affects wrinkle formation, because of weakening of the dermal-epidermal junction[45,46].

4. Senescent cell biomarkers

Cellular senescence was first described as the finite proliferative capacity of cultured normal human fibroblasts[1]. Irreversible cell growth arrest occurs due to DNA damage, telomere shortening[47], and oncogenic stress[48]. As removing senescent cells from aging tissues can delay tissue dysfunction and lead to prolonged lifespan, obvious biomarkers to identify senescent cells have been sought. The activity of β-galactosidase (β-gal) at pH 6 is increased in middle-late passage cultured fibroblasts and keratinocytes, whereas terminally differentiated keratinocytes do not express β-gal at pH 6. Activity in skin sections from the different age groups increases with age, suggesting that senescent cells accumulate *in vivo* with age[49]. Thus, the β-gal activity is termed senescence-associated β-gal (SA-β-gal) activity and remains the gold standard for identifying senescent cells in culture and in tissue samples. As senescent cells are irreversibly arrested, cell cycle regulators are usually employed to detect senescent cells. $p16^{INK4a}$, encoded by the Ink4a/Arf locus, is a tumor growth suppressor. In normal human keratinocytes, $p16^{INK4a}$, which is upregulated by single or repeated UVB irradiation, plays a role in cell cycle regulation[50]. The expression of $p16^{INK4a}$ markedly increases with advancing age in mice and humans, suggesting that $p16^{INK4a}$ is a cellular senescence marker[51,52]. A previous study showed that the number of $p16^{INK4a}$ positive cells increases with age in the skin and that numerous cardiovascular diseases are significantly associated with tertiles of $p16^{INK4a}$ positive cells in epidermal cells, suggesting an association between cell senescence and age-related pathology[53]. Previous studies have demonstrated that nuclear senescence-associated events such as heterochromatin loss, remodeling of the nuclear lamina, and DNA methylation are involved in cell proliferation[54-57]. Senescent cells secrete senescence-associated secretory phenotype (SASP) factors, including inflammatory cytokines, chemokines, MMPs and growth factors. The presence of SASP factors such as MMP3, MMP9, IL-6, IL-8, and insulin-like growth factor binding protein 7 has been used as a marker for senescent dermal fibroblasts and melanocytes[58-60]. The release of SASP factors is facilitated by the translocation of high mobility group box-1 (HMGB1) proteins from the nucleus to the cytoplasm and extracellular space in senescent cells[61,62]. While molecular hallmarks of cell senescence have been characterized *in vitro*, Lupa *et al*. demonstrated a correlation between SASP expression and age in intrinsically-aged human dermal fibroblasts, suggesting that SASP expression is upregulated along with chronological aging *in vivo*[63]. Collectively, senescent cells, which are considered passive bystanders, modulate their environment by secretion of SASP factors in both *in vitro* and *in vivo* (**Figure 1**).

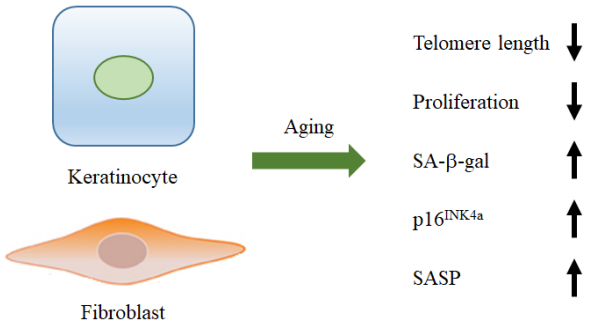

Figure 1. Senescent cell biomarkers. Shortened telomeres, reduction of cell proliferative capacity, and increased SA-β-gal activity, $p16^{INK4a}$ expression, and SASP production.

5. Inflammaging

Since Franceschi *et al.* used the term "Inflamm-aging"[64], similar terms such as "inflammaging" and various other concepts have been proposed[65-70]. Despite of confusion regarding definitions and terminology, there is consensus that inflammaging refers to low-grade, chronic, and asymptomatic inflammation, and that the primary feature of inflammaging is an increase in proinflammatory status with advancing age[71]. Several factors are involved in the underlying mechanism of inflammaging[69,72-77]. Inflammatory cytokines, which are constituents of SASP, play pivotal roles in inflammation. Previous studies have suggested that complex processes are involved in the upregulation of inflammatory cytokine expression. Although $p16^{INK4a}$ is sufficient to induce senescent cell cycle arrest[78], precipitating DNA damage leads to the upregulation of inflammatory cytokines, suggesting that the mechanism of inflammatory cytokine production is distinct from that of senescent cell cycle arrest. Studies have also demonstrated that the upregulation of inflammatory cytokine expression is triggered by activated ataxia telangiectasia mutated (ATM)-mediated DNA damage responses[79,80]. In addition to the DNA-damage response pathway, p38MAPK and protein kinase D1 are involved in the production of inflammatory cytokines. DNA-damage responses and kinases induce phosphorylation of NFκB p65/RelA subunit, followed by translocation to the nucleus where it binds to the promoters of inflammatory cytokine genes, regulating their induction during senescence[81-83]. In addition to NFκB, CCAAT/ enhancer binding protein β (CEBPβ), which is regulated by the mitogen-activated protein kinase (MAPK) pathway, participates in inflammatory cytokine production. Sebastian *et al.* showed that CEBPβ is critical for cell senescence in mouse embryonic fibroblasts[84]. Following the activation of transcription factors, it is supposed that IL-1α paracrinally regulates the production of SASP production as an upstream modulator. Previous studies have shown that IL-1α is essential for inducing the production of IL-6 and IL-8[85,86]. Consequently, low-grade chronic inflammation reinforces senescence via cell growth arrest and disruption of stem cell function[58,59,87,88]. Similar to the regulation of SASP production in intrinsic aging, previous studies have demonstrated that UVB irradiation, which is a major stimulus in extrinsic aging (photoaging), is involved in SASP regulation, such as the production of IL-6 and IL-8, activation of NFκB, activation of insulin-like growth factor-1 receptor, and HMGB1 release[89-92]. In contrast, sirtuin 1 (SIRT1), which is one of the regulators of wound healing[93], suppresses inflammatory cytokines by binding to the promoter regions of inflammatory cytokine genes[93,95]. However, because these studies were performed *in vitro*, it is necessary to perform *in vivo* experiments with a dose of UVB that meets the definition of inflammaging as asymptomatic (**Figure 2**).

6. Conclusions

Despite their different histological features and triggers, intrinsic and extrinsic aging share common biochemical mechanisms. In particular, regulation of inflammatory cytokine production is considered an important therapeutic strategy not only for acute inflammation caused by UV irradiation, but also for anti-aging. On the other hand, from a literature search regrading senescent cells, a question arose that cellular aging in the epidermis and aging in other organs should be distinguished. According to the definition of senescent cells, the cells are denoted exhibiting stable and long-term loss of proliferative capacity, and are distinguished from terminally differentiated cells. As KSCs and TA cells still have proliferative capacity, whereas senescence biomarkers are detected in the epidermis, the microenvironment surrounding KSCs and TA cells should

be addressed to explore the specific conditions involving the epidermis in the future.

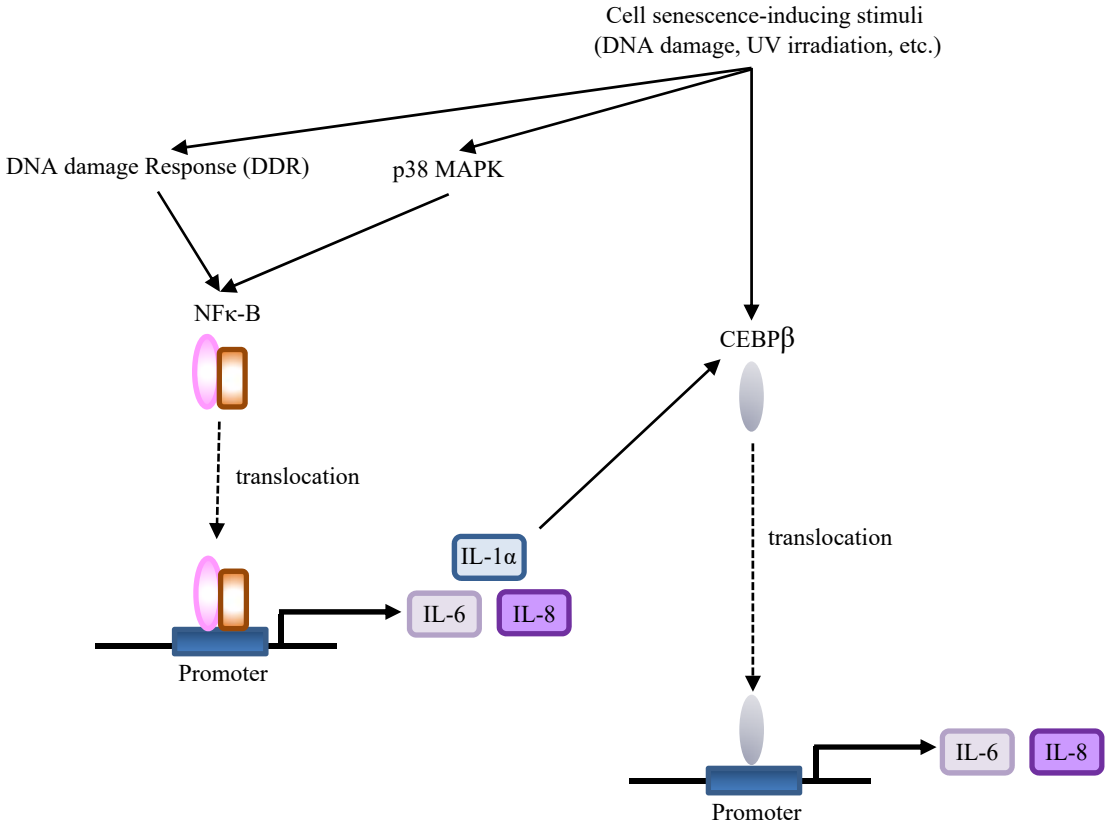

Figure 2. Inflammatory cytokine regulatory pathways. Senescence-inducing stimuli induce DDR and p38MAPK activation, followed by phosphorylation of NFκ-B. Phosphorylated NFκ-B translocates to the nucleus and then binds to the promoter regions of inflammatory cytokines genes. Consequently, the production of inflammatory cytokines is enhanced. Senescence-inducing stimuli and IL-1α, which is induced in the NFκ-B pathway, directly activate CEBPβ. Activated CEBPβ translocates to the nucleus and induces the expression of IL-6 and IL-8 expression.

Conflict of interest

There is no conflict of interest in this review.

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