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Original article

Oryza Ceramide® containing rice-derived glucosylceramides and a ceramide decreases cumulative days with cold symptoms in Japanese healthy subjects

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Abstract

***Background and objective*:** Plant-derived glucosylceramides (GlcCer) have been clinically reported to enhance skin barrier functions. GlcCer and ceramides (Cer) interact with immune-stimulating receptors on antigen-presenting cells, such as c-type lectin receptors and toll-like receptor 4. However, the clinical and immunological efficacies of orally ingested GlcCer remain unknown. Therefore, we herein conducted a clinical trial on the effects of rice-derived GlcCer (Oryza Ceramide®: OC) on cold symptoms, immune parameters, and SF-36 scores.

***Methods*:** OC (type PCD, 60 mg daily) containing 1.8 mg of GlcCer and 0.09 mg of Cer [t18:0/24:0] was used as the active sample. We enrolled 44 healthy Japanese individuals who are prone to catching colds and a low immune score. All subjects were randomly allocated to an active group (n = 22) or placebo group (n = 22). Capsules containing OC or the placebo were administered for 8 weeks. Cold symptom scores during the intervention were the primary outcome, while immune parameters and SF-36 scores measured after the 8-week intervention were the secondary outcomes.

***Results*:** Forty subjects completed the trial, and the per protocol set comprised 19 and 21 in the active and placebo groups, respectively. Regarding the primary outcome, cumulative days with cold symptoms, such as nasal congestion, throat irritation, a cough, headache, muscle pain, and diarrhea, was lower in the OC group. Among the secondary outcomes, physical functioning in SF-36 scores was improved in the OC group. Regarding immune parameters, a negative correlation was observed between changes in blood T-lymphocytes and cumulative days with cold symptoms. Laboratory tests revealed no abnormalities to suggest adverse effects of OC.

***Conclusions*:** OC consisting of GlcCer attenuated typical cold symptoms, such as nasal congestion, throat irritation, a cough, headache, muscle pain, and diarrhea, and improved physical conditions. Changes in T-lymphocytes may be one of the mechanisms by which OC ameliorates cold symptoms.

***KEY WORDS*:** rice; glucosylceramide; innate immune response; cold symptom score; T-lymphocyte

Background

Glucosylceramides (GlcCer) derived from botanical resources have been used as the ingredients of dietary supplements for skin hydration and barrier functions [1)]. Rice, wheat, pineapple, konjac potato, peach, beet, corn, and golden oyster mushroom are sources of GlcCer [2-9)]. The findings of clinical studies indicated the potential of rice-derived GlcCer to reduce transepidermal water loss [10, 11)]. Increases in epidermal ceramide (Cer) production may be one of the mechanisms by which GlcCer supplementation prevents epidermal dehydration [12)]. Other than skin-hydrating effects, recent findings demonstrated that GlcCer improved brain function [13)].

GlcCer and Cer have been reported to promote innate immune responses, particularly on antigen-presenting cells. β-GlcCer bind to macrophage-inducible c-type lectin (MINCLE) on the surface of antigen presenting cells behaving

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as if invading bacteria and fungi. Receptor recognition then induces immune responses, such as phagocytosis and inflammatory responses[14]. MINCLE also senses sterols and β-GlcCer, which are released from dead cell membranes, in addition to the innate immune defense system[15]. Moreover, MINCLE has been detected on T-lymphocytes (Th17) and its binding to β-GlcCer released from dying cells has been shown to induce inflammation in the central nervous system[16].

Whereas, α-galactosyl Cer binds to toll-like receptor (TLR)-4, which senses lipopolysaccharide (LPS) on the cell walls of Gram-negative bacteria and activates macrophages or dendritic cells to induce primary immune responses[17]. β-galactosyl Cer has also been shown to bind to TLR-4 on dendritic cells and initiate similar responses[18]. In contrast, β-GlcCer do not directly bind to TLR-4 on macrophages, but modulate the TLR-4/LPS response[19]. Cer possesses partial structural similarity to lipid A in LPS[20]. Therefore, Cer was previously proposed to induce a similar immune response to that by LPS through its binding to TLR-4, however; this hypothesis was subsequently rejected by other researchers. Although a previous study demonstrated that Cer did not bind to TLR-4, LPS increased Cer and enhanced immune responses[21]. Moreover, α-galactosylCer induced the activation of natural killer cells[22] and dendritic cells[23]. Collectively, these findings suggest that Cer derivatives induce innate immune responses, particularly in macrophages and dendritic cells; however; there is currently no clinical evidence for Cer-induced immune responses, particularly in host defenses against infections by viruses and bacteria.

Therefore, we herein conducted a clinical trial using healthy Japanese adults to investigate the effects of Oryza Ceramide® (OC) containing rice-derived GlcCer and Cer on cold symptoms and immune parameters.

Materials and methods

Subjects and grouping

All subjects were recruited between September 23 and November 20, 2021 through the Go106 website (https://www.go106.jp/) operated by ORTHOMEDICO Inc. (Tokyo, Japan). The number of subjects was assigned as the maximum number of attendees within our budget supported by the research subsidy. Inclusion criteria were healthy Japanese adults (20 years or older) who were prone to catch common cold. Exclusion criteria were as follows:

1) Currently receiving treatment for or with a previous history of cancer, heart failure, or myocardial infarction.
2) Subjects with a cardiac pacemaker or implantable cardioverter defibrillator.
3) Currently receiving treatment for arrhythmia, hepatitis, nephritis, rheumatoid arthritis, cerebrovascular disease, diabetes, hyperlipidemia, hypertension, or other chronic diseases.
4) Current use of medications or dietary supplements/beverages.
5) Subjects with allergic reactions to medicines and foods containing GlcCer.
6) Pregnancy, lactation, or expected/planned pregnancy during the study period.
7) Subjects currently participating in another clinical trial or who had participated within the previous 4 weeks.
8) Smokers.
9) Subjects who had or have respiratory diseases.
10) Subjects who have autoimmune syndromes.
11) Subjects who use immunosuppressants, such as steroids.
12) Subjects who received an influenza vaccination within 3 months.
13) Subjects considered to be inappropriate for the present study for other reasons by the attending physician.

Selection criteria were individuals with low immune scores (approximately 15) in the screening or pre-intervention periods and who were considered to be appropriate for the present study by the attending physician.

Forty-four subjects who were susceptible to the common cold and with low immune scores were selected after the confirmation of their suitability for the present study by the attending physician (***Fig. 1***). Subjects were asked to maintain the following indications.

1) Ingest 80% or more of test samples for 8 weeks.
2) Avoid excessive eating and drinking and maintain a regular lifestyle during the study period.
3) One day before testing, avoid the excessive consumption of alcohol and intensive exercise.
4) Fast for 6 hours prior to blood collection, except for drinking water.
5) Refrain from the ingestion of dietary supplements and beverages.
6) Refrain from receiving an influenza vaccination.
7) Maintain protective measures against COVID-19 infection.

Test samples and allocation

Test samples, indistinguishable brown capsules containing OC (type PCD) or placebo, were provided by Oryza Oil & Fat Chemical Co., Ltd. as hard capsules[24]. Active capsules contained 60 mg of OC-PCD (1.8 mg of GlcCer and 0.09 mg of Cer [t18:0/24:0]) and 140 mg of γ-cyclodextrin. OC-PCD consisted of 40% purified rice extract and 60% γ-cyclodextrin. Placebo capsules contained 200 mg of γ-cyclodextrin.

Oryza Oil & Fat Chemical Co., Ltd. provided the test samples with red or blue markings on the packages. Sample information was strictly concealed until the study period was complete. When the number of registered subjects reached 44, an allocation controller in Orthomedico Inc. generated an allocation sequence for test capsules according to the identification markers provided and made an allocation sheet and emergency key. Statlight #11 (Ver. 2.10, Yukms Inc.) was used to prepare a random number for the allocation sheet. The allocation sheet was only provided to test sample distributers and was then strictly concealed with the emergency key by the allocation controller. Test capsules were allocated by class randomization to equalize the allocation ratio (1:1). Allocation was performed in a manner to prevent significant differences in the means and standard deviation (SD) of Scoring of Immunological Vigor [Japan patent No. 4608704 (WO/2010/070908) and 5030109 (WO/2007/145333)], sex, and age between groups. Information on allocation was not disclosed to any other party until the subjects for analysis were selected at a clinical conference after study completion.

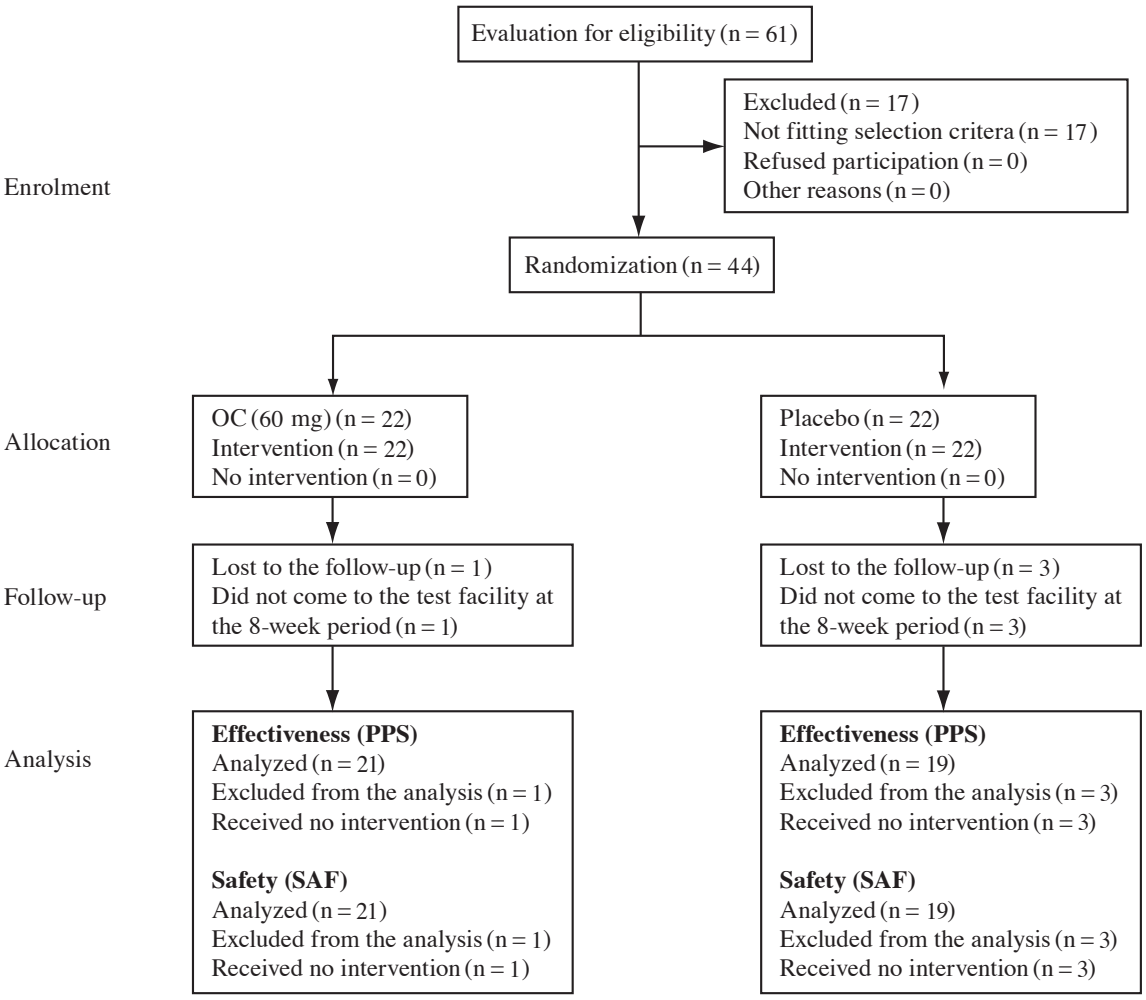

Fig. 1. **Flowchart showing subject characteristics.**

Study protocol and outcomes

This randomized, placebo-controlled, double-blind, parallel-group study was performed at Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan), and statistical analyses were conducted by Orthomedico Inc. The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000045523). Subjects took one appropriate capsule (OC or placebo) daily after breakfast for 8 weeks. All subjects recorded a daily report, including capsule ingestion, menstruation, and cold symptoms (rhinorrhea, nasal congestion, sneezing, a sore throat, throat irritation, a cough, headache, fatigue, muscle pain, diarrhea, nausea, and vomiting). They also answered a questionnaire from a physician after the 8-week ingestion period. Subjects were asked to record a Calorie And Nutrition Diary [25] from 3 days before to the day of screening.

Cold symptoms were set as the primary outcome, as previously reported [26-28], based on previously described cold symptoms in daily reports and severity was evaluated as grades 0 to 7 as follows. 0: no symptoms, 1: very mild symptoms, 2: between very mild symptoms and mild symptoms, 3: mild symptoms, 4: between mild symptoms and moderate symptoms, 5: moderate symptoms, 6: between moderate symptoms and severe symptoms, 7: severe symptoms. For the analysis, the number of days the cold symptoms appeared was counted. Secondary outcomes were Scoring of Immunological Vigor [29], the immune grade, T-lymphocyte age, T-lymphocyte count, $CD4^+/CD8^+$ T-lymphocyte ratio, naive T-lymphocyte count, ratio of naive T-lymphocytes/memory T-lymphocytes, B-lymphocyte count, natural killer NK cell count, $CD8^+/CD28^+$ T-lymphocyte ratio, and NK cell activity. In addition to these lymphocyte parameters, the SF-36 score was designated as a secondary outcome and includes a physical component summary score, mental component summary score, role/social component summary score, physical function, bodily pain, general health perception, vitality, social role functioning, role-emotional, and mental health. A higher value indicates a better health condition. These secondary parameters were examined at baseline and after the 8-week intervention.

Laboratory tests

Body weight, body mass index, the body fat ratio, blood pressure, and the pulse rate were measured before and after the 8-week intervention. Blood and urine were analyzed by LSI Medience Corporation. All items were examined at baseline and after 8 weeks of the intervention. A venous blood sample was collected from an arm vein and the following tests were performed for a safety assessment.

Hematology components were as follows: hemoglobin (Hb), hematocrit, red blood cell, leukocyte, platelet, and lymphocyte counts, and the ratios of neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Biochemical components were as follows: total protein, total bilirubin, urea nitrogen, creatinine, uric acid, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglycerides, HbA1c, blood glucose, glycoalbumin, amylase, creatine kinase (CK), aspartate aminotransferase (AST), alanine transaminase (ALT), γ-glutamyltransferase (γ-GTP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), leucine aminopeptidase (LAP), chorine esterase, Na, K, Cl, Ca, Fe, inorganic phosphorus, and immunoglobrin E (IgE).

Urine samples were collected for a qualitative evaluation, including protein, glucose, urobilinogen, bilirubin, ketone bodies, pH, and occult blood.

Ethics, adherence, and compliance

The present study was performed according to the Declaration of Helsinki (2013 revision) and conducted in conformity with ethical considerations. This protocol was approved by the Ethics Committee of Takara Clinic (Medical Corporation Seishinkai, Tokyo, Japan) on September 15, 2021 (Approved ID: 2109-00023-0115-1C-TC), and substantial deviations from the protocol required authorization by the committee. All subjects received a full explanation about the protocol and purpose of the study before consenting to participate. No subject was part of the sponsoring or funding companies.

Statistical analysis

A per protocol set was selected as the analysis dataset for the primary and secondary outcomes. Results are shown as the median and quartile or mean and SD. In statistical analyses, subject profiles, immune parameters and SF-36 scores were analyzed using Welch's *t*-test. Cumulative days with cold symptoms were analyzed by the Mann-Whitney U test. A logistic regression with backward elimination (the likelihood ratio) was performed to examine the relationship between T-lymphocytes and cumulative days with cold symptoms. The χ^2-test was used for safety parameters. We set the significance level to 5% with no adjustments for multiple comparisons. SPSS (Ver. 23.0, Japan IBM) was employed for statistical evaluations. Missing data were analyzed without storage.

Results

Study performance

The present study was performed between December 7, 2021 and February 19, 2022. During the study period, one subject in the test group and 3 in the placebo group were unable to come to the test facility after the 8-week intervention for personal reasons (***Fig. 1***), and, thus, were excluded from the analysis from this time point. Accordingly, 21 subjects (42.1 ± 14.4 years) in the OC group and 19 (42.8 ± 11.7 years) in the placebo group were available for analysis. The physical profiles of subjects included in the analysis are shown in ***Table 1***. No significant differences were observed between the groups.

Cold symptom scores

After the 8-week intervention, cumulative days with mild to severe cold symptoms (the total cold score) were lower in the OC group than in the placebo group (***Table 2***). Similarly, the severities of nasal congestion, throat irritation,

Table 1. Profiles of subjects.

	Baseline		8 W	
	OC	Placebo	OC	Placebo
Age	42.1 ± 14.4	42.8 ± 11.7	–	–
Height (cm)	162.7 ± 8.1	165.1 ± 9.7	–	–
Body weight (kg)	59.3 ± 11.3	63.7 ± 15.0	52.3 ± 7.7	54.4 ± 9.6
BMI (kg/m^2)	22.4 ± 4.3	23.1 ± 3.7	21.1 ± 3.0	21.7 ± 3.6
Body fat ratio (%)	26.2 ± 10.4	27.1 ± 6.6	27.7 ± 7.7	28.8 ± 7.4
Systolic blood pressure (mmHg)	120.4 ± 14.8	120.4 ± 20.0	114.2 ± 12.3	115.6 ± 13.1
Diastolic blood pressure (mmHg)	79.6 ± 9.4	78.8 ± 14.5	72.4 ± 9.1	76.2 ± 11.4
Pulse rate (bpm)	74.6 ± 11.7	71.2 ± 10.5	69.1 ± 8.9	74.0 ± 7.7

Data are shown as the mean ± SD (n = 22 for baseline, n = 21 for OC and n = 19 for the placebo at the 8-week period). Welch's *t*-test was used to assess the significance of differences, except for age (the χ^2-test). No significant differences were detected between the placebo and OC groups. OC, Oryza Ceramide®; BMI, body mass index; SD, standard deviation.

Table 2. Cumulative days with cold symptoms.

	Severity	OC	Placebo
Total cold score	Very mild to severe symptoms	35.0 (7.0-46.0) Min: 0.0/Max: 56.0	44.0 (38.0-55.0) Min: 4.0/Max: 66.0
	Mild to severe symptoms	1.0 (0.0-10.0)* Min: 0.0/Max: 44.0	15.0 (3.0-27.0) Min: 0.0/Max: 46.0
	Moderate to severe symptoms	0.0 (0.0-1.0) Min: 0.0/Max: 16.0	0.0 (0.0-6.0) Min: 0.0/Max: 19.0
Rhinorrhea (yellow secretion)	Very mild to severe symptoms	1.0 (0.0-17.0) Min: 0.0/Max: 56.0	9.0 (0.0-18.5) Min: 0.0/Max: 66.0
	Mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 31.0	0.0 (0.0-1.5) Min: 0.0/Max: 24.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.0) Min: 0.0/Max: 2.0
Rhinorrhea (red secretion)	Very mild to severe symptoms	0.0 (0.0-1.0) Min: 0.0/Max: 56.0	0.0 (0.0-0.5) Min: 0.0/Max: 44.0
	Mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 28.0	0.0 (0.0-0.0) Min: 0.0/Max: 5.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 0.0	0.0(0.0-0.0) Min: 0.0/Max: 3.0
Nasal congestion	Very mild to severe symptoms	5.0 (0.0-25.0) Min: 0.0/Max: 56.0	27.0 (5.0-42.5) Min: 0.0/Max: 64.0
	Mild to severe symptoms	0.0 (0.0-0.0)* Min: 0.0/Max: 28.0	4.0 (0.0-14.5) Min: 0.0/Max: 30.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.0) Min: 0.0/Max: 14.0
Sneezing	Very mild to severe symptoms	7.0 (0.0-20.0) Min: 0.0/Max: 56.0	11.0 (7.0-35.0) Min: 0.0/Max: 62.0
	Mild to severe symptoms	0.0 (0.0-1.0) Min: 0.0/Max: 31.0	1.0 (0.0-4.5) Min: 0.0/Max: 16.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.0) Min: 0.0/Max: 2.0
Sore throat	Very mild to severe symptoms	4.0 (0.0-10.0) Min: 0.0/Max: 26.0	14.0 (0.0-22.0) Min: 0.0/Max: 56.0
	Mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 5.0	0.0 (0.0-6.0) Min: 0.0/Max: 16.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.5) Min: 0.0/Max: 5.0
Throat irritation	Very mild to severe symptoms	1.0 (0.0-6.0) Min: 0.0/Max: 34.0	11.0 (0.0-19.0) Min: 0.0/Max: 54.0
	Mild to severe symptoms	0.0 (0.0-0.0)* Min: 0.0/Max: 6.0	0.0 (0.0-3.5) Min: 0.0/Max: 17.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0,0 (0.0-0.0) Min: 0.0/Max: 3.0
Cough	Very mild to severe symptoms	0.0 (0.0-8.0)* Min: 0.0/Max: 56.0	15.0 (2.0-30.0) Min: 0.0/Max: 56.0
	Mild to severe symptoms	0.0 (0.0-0.0)* Min: 0.0/Max: 44.0	1.0 (0.0-4.5) Min: 0.0/Max: 21.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 15.0	0.0 (0.0-0.0) Min: 0.0/Max: 4.0
Headache	Very mild to severe symptoms	3.0 (0.0-6.0)* Min: 0.0/Max: 33.0	10.0 (3.0-17.5) Min: 0.0/Max: 45.0
	Mild to severe symptoms	0.0 (0.0-2.0) Min: 0.0/Max: 7.0	1.0 (0.0-2.5) Min: 0.0/Max: 20.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 2.0	0.0 (0.0-0.5) Min: 0.0/Max: 7.0
Fatigue	Very mild to severe symptoms	2.0 (0.0-9.0) Min: 0.0/Max: 52.0	5.0 (1.0-27.0) Min: 0.0/Max: 56.0
	Mild to severe symptoms	0.0 (0.0-1.0) Min: 0.0/Max: 19.0	1.0 (0.0-8.0) Min: 0.0/Max: 26.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 3.0	0.0 (0.0-1.0) Min: 0.0/Max: 7.0

Muscle pain	Very mild to severe symptoms	0.0 (0.0-2.0)* Min: 0.0/Max: 49.0	3.0 (0.5-18.5) Min: 0.0/Max: 42.0
	Mild to severe symptoms	0.0 (0.0-0.0)* Min: 0.0/Max: 2.0	0.0 (0.0-3.0) Min: 0.0/Max: 21.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 0.0	0.0 (0.0-0.0) Min: 0.0/Max: 5.0
Diarrhea	Very mild to severe symptoms	0.0 (0.0-1.0)** Min: 0.0/Max: 9.0	3.0 (0.0-14.5) Min: 0.0/Max: 39.0
	Mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 3.0	0.0 (0.0-4.0) Min: 0.0/Max: 20.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.5) Min: 0.0/Max: 5.0
Nausea	Very mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 18.0	0.0 (0.0-1.5) Min: 0.0/Max: 34.0
	Mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.5) Min: 0.0/Max: 15.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.0) Min: 0.0/Max: 4.0
Vomiting	Very mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 3.0	0.0 (0.0-0.0) Min: 0.0/Max: 22.0
	Mild to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.0) Min: 0.0/Max: 10.0
	Moderate to severe symptoms	0.0 (0.0-0.0) Min: 0.0/Max: 1.0	0.0 (0.0-0.0) Min: 0.0/Max: 1.0

Days with cold symptoms are shown as the median and quartile and minimum and maximum values (n = 21 for OC and n = 19 for the placebo). Data were analyzed by the Mann-Whitney U test. Asterisks indicate significant differences from the placebo group at *: $p < 0.05$. **: $p < 0.01$, respectively. OC, Oryza Ceramide®.

a cough, and muscle pain were significantly lower in the OC group. Cumulative days with very mild to severe coughing, headache, muscle pain, and diarrhea were also significantly lower in the OC group.

Immune scores and parameters

After the 8-week intervention, immune scores, T-lymphocyte age, and NK cell activity did not significantly differ between the OC and placebo groups (***Table 3***). The numbers of T-lymphocyte species, B-lymphocytes, and NK cells were not affected by the OC intervention. A negative correlation was observed between changes in blood T-lymphocytes and cumulative days with cold symptoms in the OC group (***Fig. 2***).

SF-36 scores

Table 4 shows the SF-36 scores of subjects after the 8-week intervention. Only physical functioning was significantly enhanced in the OC group.

Laboratory data and adverse effects

Blood pressure, the pulse rate, and body temperature are shown in ***Table 1***. No significant differences were observed between the groups. The number of subjects with values outside of normal ranges is shown in ***Table 5*** (blood hematology parameters) and ***Table 6*** (blood biochemical parameters). As shown in ***Table 6***, the number of the subjects who had LDL-C out side of normal range in the OC group were lower than the placebo group after the 8-week intervention. Urinalysis parameters did not significantly change in either group (***Table 7***).

Discussion

Primary care and fork remedies against cold

In the early stages of the common cold, individuals purchase over-the-counter cold drugs or are prescribed medicines. However, a recent study[30] reported that 90% of prescriptions for the common cold, such as β_2-stimulants and cephem-type anti-bacterial medicines, are inappropriate. Inaccurate diagnoses and inappropriate prescriptions by physicians sometimes cause serious adverse effects and prolong recovery. Therefore, safer and supportive remedies to cure cold symptoms are required. In China, specific prescriptions of Chinese medicines to treat the common cold are selected based on symptoms[31]. Chinese prescriptions consist of several natural Chinese medicines and have been used and improved for thousands of years. A physician considers the characteristics, symptoms, and changes in the appearance of the tongue of patients, and selects the appropriate prescription. Therefore, the incidence of side effects is lower than with commonly used synthetic medicines.

Multivitamins and minerals are preferably taken as personal care to prevent the common cold[32], and zinc has been shown to reduce the severity of cold symptoms[33]. Echinacea has traditionally been used as a herbal remedy against the common cold. A meta-analysis of 10 clinical studies on echinacea revealed that the incidence and duration of colds were improved by 58% and –1.4 days, respectively[34]. Echinacea has also been suggested to effectively ameliorate cold symptoms, particularly the duration; however, a higher dosage, 100 to 900 mg equivalent of echinacea, was required than rice GlcCer (1.8 mg). As probiotics, the ingestion of *Lactobacillus paracasei* MCC1849 for between 6 and 12 weeks reduced the number of days of infection and symptoms in subjects with cold symptoms in the previous year[35].

Table 3. Immune scores and blood immune parameters at the 8-week period

	OC	Placebo
Immune scores	14.4 ± 1.7	14.7 ± 1.4
T-lymphocyte age (year)	54.6 ± 11.8	54.8 ± 11.0
T-lymphocytes (/μL)	1068 ± 245	1195 ± 181
CD^{4+}/CD^{8+} ratio	8.8 ± 17.0	7.6 ± 10.1
Naive T-lymphocytes (/μL)	251 ± 105	286 ± 120
Naive/memory cell ratio	0.6 ± 0.3	0.6 ± 0.3
B-lymphocytes	278 ± 111	268 ± 145
NK cells	214 ± 149	213 ± 80
$CD^{8+}CD^{28+}$ lymphocytes	122 ± 65	127 ± 83
NK cell activity	53.7 ± 21.2	50.2 ± 23.4

Each value is shown as the mean and SD (n = 21 for OC and n = 19 for the placebo). Data were analyzed using Welch's *t*-test. No significant difference was observed from the placebo group. OC, Oryza Ceramide®; NK, natural killer; SD, standard deviation.

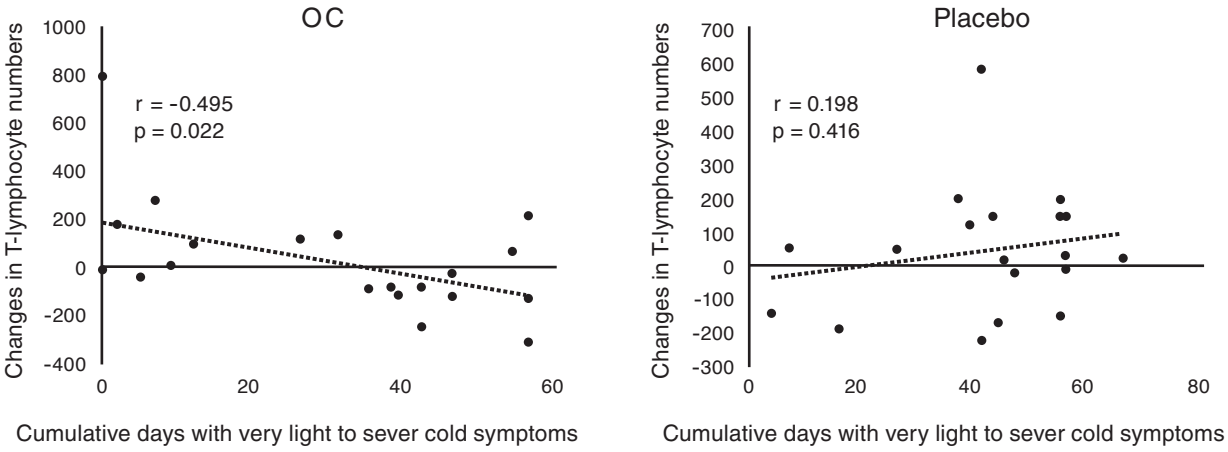

***Fig. 2.* Correlations between changes in the numbers of T-lymphocytes in the 8-week period and cumulative days with very light to severe cold symptoms.**

A logistic regression with backward elimination (likelihood ratio) was employed. OC, Oryza Ceramide®.

Table 4. Changes in SF-36 parameters at the 8-week period.

	OC	Placebo
PCS	54.3 ± 5.8	52.6 ± 6.2
MCS	54.1 ± 9.0	49.8 ± 8.3
RCS	51.1 ± 6.7	49.9 ± 8.7
Physical functioning	96.2 ± 4.4*	92.1 ± 9.3
Role function	93.5 ± 12.4	87.5 ± 13.6
Bodily pain	83.3 ± 19.6	75.7 ± 17.6
General health perception	68.2 ± 14.3	62.8 ± 13.2
Vitality	65.8 ± 18.2	55.3 ± 17.3
Social role functioning	85.1 ± 21.5	83.6 ± 18.7
Role-emotional	94.8 ± 10.4	86.8 ± 15.3
Mental health	76.9 ± 15.6	67.4 ± 19.1

Scores are shown as the mean and SD (n = 21 for OC and n = 19 for the placebo). Data were analyzed using Welch's *t*-test. An asterisk indicates a significant difference from the placebo group at *: $p < 0.05$. OC, Oryza Ceramide®; PCS, physical component summary; MCS, mental component summary; Role/Social component summary; SD, standard deviation.

Table 5. Number of subjects with hematological values outside of normal ranges.

	OC (n = 21)	Placebo (n = 19)
Red blood cells	1	0
Leukocytes	0	0
Hemoglobin	3	0
Hematocrit	1	0
Platelets	2	0
Neutrophil ratio	0	2
Lymphocyte ratio	0	1
Monocyte ratio	1	0
Eosinophil ratio	0	0
Basophil ratio	0	0

Each value is shown as the number of subjects with values outside of the standard value after the intervention. The χ^2-test was used for statistical analyses. No significant differences were detected between the placebo and OC groups. OC, Oryza Ceramide®.

Table 6. Number of subjects with blood biochemical parameter values outside of normal ranges.

	OC (n = 21)	Placebo (n = 19)
Total protein	1	1
Total bilirubin	0	1
Urea Nitrogen	2	1
Creatinine	2	0
Total cholesterol	0	1
LDL-C	0*	5
HDL-C	0	2
Triglycerides	1	2
HbA1c	0	1
Glycoalbumin	3	2
Blood glucose	0	0
Amylase	2	1
CK	0	0
AST	0	1
ALT	0	0
γ-GTP	0	0
ALP	0	0
LAP	1	1
LDH	1	2
Na	0	0
K	4	3
Cl	0	0
Ca	0	0
Fe	1	1
Inorganic P	0	2

Each value is shown as the number of subjects with values outside of the standard value after the intervention. The χ^2-test was used for urinalysis parameters. An asterisk denotes a significant difference between the placebo and OC groups at *: $p < 0.05$. OC, Oryza Ceramide®. For other abbreviations, see “Laboratory tests” in methods.

Table 7. Number of subjects with urinalysis values outside of normal ranges.

	OC (n = 21)	Placebo (n = 19)
Protein	2	3
Glucose	0	0
Urobilinogen	0	0
Bilirubin	0	0
pH	0	0
Occult blood	0	2
Ketone bodies	0	0

Each value is shown as the number of subjects with values outside of the standard value after the intervention. The χ^2-test was used for statistical analyses. No significant differences were detected between the placebo and OC groups. OC, Oryza Ceramide®.

Involvements of GlcCer and Cer on immune system

In the present study, we examined the clinical efficacy of a rice GlcCer and Cer composition (OC) against cold symptoms and immune parameters in healthy subjects. We selected these parameters as outcomes for the following reasons. Between 80 and 90% of common colds in Japan are viral-induced acute respiratory infections[36]. When a virus infects the mucous membrane of the upper respiratory tract, various cytokines are produced as a biological reaction, causing symptoms such as nasal discharge, a sore throat, cough, and general malaise[36]. One approach to alleviate cold symptoms is to regulate the innate immune system in order to enhance immune responses and suppress viral invasion and proliferation[36].

Cer, an aglycon of GlcCer, has been shown to regulate dendritic cells, neutrophils, and macrophages, which are involved in innate immunity[37]. In addition, Cer plays a role in the maturation of and expression of antigens on dendritic cells and is an important structural element of lipid rafts in the cell membrane of dendritic cells, which is required for virus binding, uptake, and internalization as well as membrane stabilization. In addition, the cell membrane is responsible for important processes, such as antigen uptake, virus internalization, viral peptide processing, and presentation to T-lymphocytes to eliminate virus-infected cells. These findings strongly suggest that Cer is a major effector of dendritic cell function[37]. In addition to regulating the production of active oxygen species in neutrophils, Cer has been suggested to contribute to the regulation of neutrophil phagocytosis and migration[37]. Furthermore, Cer was found to regulate macrophage function and metabolism in the early and late stages of infection[37].

Clinical efficacy of OC on cold symptoms

Therefore, we selected cold symptom scores and immune parameters as outcomes and examined the immunomodulatory function of GlcCer and Cer in healthy adult Japanese men and women who were susceptible to the common cold. The results obtained showed that cumulative days with "mild to severe" cold symptoms during the study period were significantly lower in the OC group than in the placebo group (***Table 2***). Furthermore, cumulative days with "mild to severe" nasal congestion, throat irritation, coughing, and muscle pain were significantly lower in the OC group than in the placebo group. Cumulative days with "very mild to severe" coughing, headache, muscle pain, and diarrhea were significantly lower in the OC group than in the placebo group.

Primary outcome

We used the cold symptom score as a primary outcome in the present study. This scoring system is more reasonable than counting the number of virions and other biological scores because it reflects similarities among virus induced cold symptoms and pneumonia[38]. However, it cannot accurately distinguish other respiratory diseases, such as rhinitis[39]. Therefore, this system is both advantageous and disadvantageous. As a representative respiratory infection, COVID-19 is still affecting our society. The typical symptoms of COVID-19 differ from those of influenza and the common cold. Patients with COVID-19 commonly present with a cough (70%), fever (45%), muscular pain (29%), and headache (21%), with lower rates of a sore throat (12%) and rhinorrhea (4%)[27]. A sore throat was rarely reported in COVID-19 and SARS (12% and 18%, respectively). In influenza and the common cold, a cough was identified in 93 and 80% of cases. Headache, rhinorrhea, muscular pain, and a sore throat were more common in patients with influenza and the common cold[27]. In the present study, subjects developed nasal congestion, throat irritation, a cough, headache, muscle pain, and diarrhea, which were attenuated by the OC intervention. The duration of the intervention was for 2 months between December 2021 and February 2022. At that time, the number of newly infected COVID-19 patients with Omicron variants markedly increased. Therefore, OC appeared to improve symptoms in subjects with influenza and the common cold instead of COVID-19. During the study period, all subjects wore a mask; however, this did not prevent influenza or the common cold. Jacobs *et al.*[40] previously reported that wearing surgical masks did not prevent the common cold. Only a few studies demonstrated the preventive effects of face masks on infection by influenza[41]. Based on these findings, we did not expect the wearing of masks to prevent infection by the common cold or influenza.

Cer belongs to the sphingolipid family, and dietary sphingolipids have been suggested to interact with the immune system of a host [42]. *In vitro* and *in vivo* studies showed that sphingolipids exerted antibacterial effects against Gram-positive and -negative pathogenic bacteria [43-45]. Furthermore, preventive effects of sphingolipids involving innate and immediate defense mechanisms in multiple epithelial tissues against severe infectious diseases were confirmed [46]. Moreover, sphingosines metabolized from Cer were highly expressed in human nasal epithelial cells and were associated with a barrier effect. Bacterial infection has been shown to decrease sphingosine levels [47]. In contrast, phosphorylated sphingosines affect infectious dynamics through the migration and differentiation of immune cells and also contribute to maintaining barrier functions [48, 49]. Orally ingested GlcCer and Cer are converted to a sphingoid base and absorbed into intestinal membranes. They are then resynthesized into Cer and enter lymph ducts [8]. Therefore, Cer and its metabolites derived from OC have been suggested to attenuate various cold symptoms, such as a sore throat and diarrhea, by suppressing the early stage of infection by pathogens.

No significant differences were observed in the immune parameters of secondary outcomes between the OC and placebo groups (***Table 3***). The functions of immune cells [50] and cytokine production [51] are affected by aging and the immune system differs among individuals and with each season [50]. Nevertheless, we found a negative correlation between T-lymphocytes and cumulative days with cold symptoms in the OC group (***Fig. 2***). The regulation of T-lymphocyte responses by OC may be one of mechanisms suppressing cold symptoms. Therefore, in further trials on the effects of OC on immune parameters, the consideration of these findings in recruitment criteria for subjects and their allocation will contribute to more accurate evaluations.

Secondary outcome

Regarding the other secondary outcomes, OC significantly improved physical functioning in the SF-36 score (***Table 4***) [52]. In the present study, the slight or significant suppression of the onset of cold symptoms was observed for fatigue with "severe symptoms" ($p = 0.098$) and muscle pain with "very mild symptoms to severe symptoms" ($p < 0.05$). Therefore, inhibiting the onset of these cold symptoms may have improved physical functioning and ultimately maintained the quality of life of subjects.

Safety

In the safety assessment, no side effects were observed during the intervention under the conditions of the study; however, several side events were noted in some subjects (data not shown). Based on criteria set at the time of study planning, the investigator indicated no causal relationship with OC. The number of subjects with higher or lower LDL cholesterol than the standard value was significantly higher in the placebo group. However, the investigator analyzed each item on an individual basis and confirmed no medically problematic changes associated with the intake of OC or placebo. Therefore, the ingestion of OC under the conditions of the present study was safe.

Conclusions

The present study demonstrated that Oryza Ceramide® (OC), 60 mg/day for 8 weeks, containing 1.8 mg of GlcCer and 0.09 mg of Cer[t18:0/24:0] ameliorated cold symptoms and improved role functioning in the SF-36 score. A correlation was observed between changes in the number of T-lymphocytes and cumulative days with cold symptoms in the OC group, suggesting the prevention of the common cold due to an increase in the number of T-lymphocytes. Therefore, the ingestion of rice-derived GlcCer may prevent infection by the common cold and influenza through immune responses, such as T-lymphocytes. The intake of OC was safe under the conditions of the present study.

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Conflict interest

The sponsor of the present study, Oryza Oil & Fat Chemical Co., Ltd., assigned Orthomedico Inc. to conduct the study. H.S. (Ph.D.) and W.Y. are affiliated with Oryza Oil & Fat Chemical Co., Ltd., and K.Y., N.S., S.I., H.N., T.K., and A.B. are members of Orthomedico Inc. This study was conducted by both Oryza Oil & Fat Chemical Co., Ltd. and Orthomedico Inc. T.T. (MD) was the principal investigator who monitored the condition of all subjects. MN (MD) supported TT. W.Y., prepared GlcCer capsules and measured GlcCer contents.

Author contributions

Conceptualization: H.S. and T.T. Data curation: H.N. and T.K. Formal analysis: T.K. Funding acquisition: H.S. Investigation H.N., T.K., A.B., and T.T. Methodology: K.Y., N.S., and S.I. Project administration: K.Y., N.S., and T.T. Resources: K.Y., N.S., TT, W.Y., and S.T. Supervision: T.T. and M.N. Visualization: T.K., A.B., and H.S. Writing-original draft H.S. Writing-review and editing: K.Y., N.S., S.I., A.B., and H.S.

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