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The Effect of Intake of Gliadin-Combined Superoxide Dismutase (SOD)-rich Melon Extract (GME) on Minimal Erythema Dose (MED): A Randomised, Double-blind, Placebo-controlled, Parallel-group Study of Healthy Japanese.

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Gliadin-combined superoxide dismutase (SOD)-rich Melon Extract (GME, trade name: GliSODin®, ISOCELL Nutra S.A.S, France) has already demonstrated activation properties on the principal internal antioxidant enzymes, particularly SOD, Catalase and Glutathione Peroxidase. The purpose of this study was to assess the efficacy of GME in helping increase the Minimal Erythema Dose (MED) which plays a role in preventing sunburn. Forty-one healthy Japanese male subjects of skin phototypes II and III took either 250 mg / day of GME capsules or placebo capsules for eight weeks. The intake of GME induced a significant increase in the MED (5.1% versus -5.0% for the placebo group). This improvement in sun protection might be attributable to the increased antioxidative capacity induced by the intake of GME.

Keywords: gliadin-combined SOD-rich melon extract (GME) / minimal erythema dose (MED) / sunburn / sun protection / superoxide dismutase (SOD)

Introduction

Solar rays affect the skin both beneficially and adversely. Mild exposure such as from brief sunbathing promotes vitamin D production, which is essential to bone formation, while excessive skin exposure to sunlight induces acute inflammation (sunburn), eventually leading to delayed tanning (Masaki, 2013). Reactive oxygen species, formed in the skin by solar ultraviolet radiation, play a major role in the damage to DNA and cell membranes associated with sunburn (Kamide, 2007). Since sunburn may cause health and cosmetic problems, it is important for efficient elimination of reactive oxygen species generated in the body by ultraviolet irradiation.

Oral consumption of the complex of wheat (*Triticum aestivum*) gliadin and a superoxide dismutase (SOD)-rich melon (*Cucumis melo*) extract, known as Gliadin-combined SOD-rich Melon Extract (GME) and marketed under the trade name of Melon GliSODin ® (ISOCELL Nutra S.A.S., France), has been shown to increase SOD, catalase, and glutathione peroxidase activity levels in serum and the liver, enhancing antioxidant functions in the body (Vouldoukis et al., 2004). A study in France examined the effect of GME given orally for 4 weeks to 30 Caucasian subjects with skin photo types II (burns [reddens] easily and tans minimally) and III (burns [reddens] and then tans readily), and demonstrated the improvement of sunburn resistance as indicated by an increase in the minimal erythema dose (MED) (Mac-Mary et al., 2007). A similar study in South Korea suggested that the oral consumption of GME increased MED (Cho, 2009). These studies, however, did not report the extent of MED increases in individuals. It is not known whether or not the oral consumption of GME is effective in MED enhancement in the Japanese. The present study evaluated the effect of oral GME on MED in Japanese subjects.

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Methods

1. Ethics

This study was approved in advance by the Ethics Review Board of Shirasawa Clinical Research Center and was conducted based on the principles stipulated in the Declaration of Helsinki, respecting the rights of the subjects, and in compliance with the national ethical guidelines for medical research in humans (Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, Japan). The principal investigator (and his assistants) gave sufficient explanations to prospective subjects before their participation in the study (before the baseline examination) using the explanation and consent forms approved by the Ethics Review Board, confirmed that they fully understood and agreed to the details of the study, and enrolled them as study subjects after they provided written consent by their own free will. The study was conducted from December 6, 2015 to February 20, 2016.

2. Subjects

The subjects were 70 healthy males aged ≥20 and <50, invited

principally via the Internet, who self-reported to be of the skin photo type II or III and voluntarily provided written consent to participate in the study. We excluded the individuals falling under any of the exclusion criteria: 1) a diagnosis of photosensitivity; 2) the use of medication that may affect the results of the examination (e.g., corticosteroid oral medication, injection, or suppository); 3) the routine use of functional skin-care products (whitening, spot removal, sagging treatment, moisturising, etc.) or functional foods (not limited to supplements) that may affect the results of the examination; 4) excessive alcohol consumption; 5) a risk of allergy to wheat, melon, or latex or a risk of other oral allergies (apple, banana, peach, carrot, soybean, watermelon, peanut, potato, or tomato); 6) current hospital care for dermatological conditions; 7) the presence of factors that may affect the results of the examination in the skin area used for evaluation (disorders such as atopic dermatitis and urticaria, conditions such as inflammation, eczema, trauma, acne, eruptions, warts, spots, etc., or vestiges of these conditions); 8) subjective symptoms of severe anemia; 9) intervention provided by medical services for treatment or prevention of a disease (hormone replacement therapy, medical therapy, therapeutic exercise, dietary therapy, etc.) or a condition considered to require such intervention; 10) current or past history of serious disorders involving carbohydrate metabolism, lipid metabolism, liver function, kidney function, the heart, the circulatory system, the respiratory system, the endocrine system, or the nervous system, as well as a psychiatric disease or alcohol or drug dependence (including a positive test for the hepatitis virus); 11) skin care treatment (aesthetic salon services, aesthetic treatment, application of cosmetics, etc.) or massage affecting the skin area used for an evaluation performed in the past four weeks or planned to be performed during the study period; 12) high exposure to ultraviolet rays exceeding the normal amount in daily living, such as from long outdoor work, sports, sea bathing and/or sunbathing, tanning salons, etc., occurring in the past two months or expected to occur during the study period (including travelling to areas with high ultraviolet exposure and overseas locations); 13) irregular or disturbed circadian rhythm resulting from night work, alternate day and night shifts, etc.; 14) participation in another clinical study (including all studies on humans using cosmetics, foods, medicines, non-medicinal items, medical devices, etc.) or a plan to participate in another clinical study during the planned period of this study; 15) habitual smoking; 16) difficulty in maintaining routine life habits (eating, exercise, sleep, skin care, etc.) during the study period including the New Year holidays; 17) difficulty in keeping the study diary; and 18) the subjects who the principal investigator considers inappropriate for the study.

During the study period, the subjects were asked to comply with the following restrictions and prohibitions: 1) During the study period, the subjects must maintain the same habits as before their participation in the study in terms of eating, exercise, drinking, smoking, sleep, etc., without changes (restriction). 2) During the study period, the subjects must avoid excessive exercise, lack of sleep, dieting, and immoderate eating and drinking significantly deviating from the normal range (restriction). 3) During the study period, the subjects must take care to avoid excessive ultraviolet exposure exceeding the normal amount in daily living (restriction). 4) During the study period, medication must not be used unless there is an unavoidable need, and any use of medication must be recorded in the diary, providing the name and dose of the medication (restriction). 5) During the study period, cosmetic medical care and special skin care (such as aesthetic treatment) are prohibited (prohibition). 6) During the study period, the subjects must not rub their backs forcefully at any times, including while bathing (prohibition). 7) Any healthy foods and non-medicinal items consumed regularly before participation in the study must be continued without changing the amount, frequency, and method of consumption, while consumption of a new non-medicinal item or healthy food is prohibited (prohibition). 8) During the period of three days before the date of each examination, the subject must not apply strong pressure, rubbing, or stimulation to their backs, must not sit up late at night or all night, and must not engage in strenuous physical activities (running, swimming, mountain climbing, etc., causing breathlessness) (prohibition). 9) The subjects must take a bath before going to bed on the day before the date of each examination, and must not take a bath (including a shower) until the end of the examination on the next day (prohibition). 10) On the day before the date of each examination, the subjects must avoid alcohol consumption, ensure sufficient sleep, and stay in good physical condition. 11) When the subjects visit the laboratory on the day of each examination, they must avoid behaviour that may affect their skin, such as running and fast walking, even if they are hurrying to arrive on time.

3. Test Food Product

The test product, GME, was a hard-capsule preparation containing GliSODin® CM01 powder (ISOCELL Nutra S.A.S., France) consisting of a melon fruit extract, wheat protein (gliadin), maltodextrin derived from wheat, and trace amounts of carboxymethylcellulose sodium and glycerin fatty acid ester (Table 1). Each capsule contained 83.4 mg of GliSODin® as the active component, in addition to dextrin and calcium stearate as a vehicle (Table 1). The control product (placebo) was a hard-capsule preparation lacking GliSODin®, which was identical in appearance to the test product (Table 1). The dose was set at 3

Table 1 Composition of a GME capsule and a placebo capsule

		GME capsule (mg)	Placebo capsule (mg)
GliSODin® CM01 powder		83.4	0.0
	Contents (%)		
Maltodextrin	95.72		
Wheat gliadin	2.70		
Melon extract	0.95		
Carboxymethyl cellulose sodium	0.60		
Glycerin fatty acid ester	0.03		
Dextrin		64.6	118.0
Calcium stearate		2.0	2.0
Total		150.0	120.0

capsules/day based on the information from previous studies (Mac-Mary et al., 2007; Cho, 2009). With respect to the toxicity of GliSODin®, a 28-day oral toxicity study in rats (2,000 mg/kg/day) (internal data, ISOCELL Nutra S.A.S., France) and a 2-year oral intake study in humans (500 mg/day) (Cloarce et al., 2007) demonstrated no abnormalities. In addition, Melon GliSODin® is a food ingredient that has been marketed in Japan and other countries for more than 10 years. Although it is not a food consumed commonly, all of its raw materials are supported by a large body of experience in the use for human consumption. The product is, therefore, considered safe.

4. Procedures

4.1 Screening

On the first day of the screening test, prospective subjects were given an explanation of the purpose and details of the study, and the screening test was performed on the individuals who provided written consent. The measurement of MED was performed on the back of each subject, which was an area considered to receive relatively little sunlight in daily living. In the preliminary irradiation 1, the back of each subject was irradiated with ultraviolet light at 6 dose levels defined by a common ratio of 1.15 around the expected 1 MED. Each irradiation field had an area of 0.5 cm^2 (ϕ 8 mm). On the next day (second day of the screening test), the areas irradiated in preliminary irradiation 1 were inspected to determine the 1 MED. As a result, 44 subjects were selected for the main experiment. The ultraviolet light source used in the study was a xenon arc solar simulator (DRC Co., Ltd., Osaka), calibrated according to the Standards for SPF Measurement (Japan Cosmetic Industry Association, 2007). MED was calculated as the product of the ultraviolet light intensity (mW/cm^2) and the duration of irradiation (sec). Other test items included subject background (medical history, allergy status, skin condition, skin care status, cosmetic medicine and aesthetic treatment status, medication and healthy food consumption status), blood tests (hepatitis virus [HBs antigens, HCV antibodies], specific IgE tests [melon, wheat, latex, apple, banana, peach, carrot, soybean, watermelon, peanut, potato, tomato], general biochemistry [AST, ALT, γ-GTP, total bilirubin, total protein, creatinine, urea nitrogen, Na, K, Cl, blood glucose, HbA1c, total cholesterol, LDL cholesterol], hematology [white blood cells, red blood cells, hemoglobin, hematocrit, platelets]), and urinalysis.

4.2 Main Experiment

The study was designed as a randomised placebo-controlled double-blind parallel-group comparison study. Each subject selected for the study was provided with a diary on the first day of the preparation period. Either the test product or the placebo product was handed out 8 days after (on the eighth day of the preparation period), and consumption started from the night of the same day. The subjects visited the laboratory 56 days after the start of consumption, and the measurement of MED was performed in the same manner as in the screening test. The subjects also visited the facility 30 days after the start of consumption so that we could check consumption and diary keeping. The subjects were asked to keep the diary to record the consumption status of the test product (whether or not it was consumed and the time of consumption), health problems, the use of medications and supplements, and unusual events (such as business trips) every day until the end of the study period.

Table 2 Characteristics of the subjects

	GME group (n=21)	Placebo group (n=20)
Age (years)	37.1 ± 9.6	39.4 ± 8.4
MED at 0 week (mJ/cm^2)	29.2 ± 5.9	30.0 ± 6.1

Values are mean ± SD. No significant difference is found.

Table 3 Change in MED by the intake of GME

MED	GME group	Placebo group
0 week (mJ/cm^2)	29.2 ± 5.9 (100.0)	30.0 ± 6.1 (100.0)
8 weeks (mJ/cm^2)	30.7 ± 5.6 (105.1) *	28.5 ± 6.6 (95.0)
⊿8weeks (mJ/cm^2)	1.5 ± 3.0 **	-1.5 ± 4.0

Values are mean ± SD. Values in parenthesis are relative values to the 0 week level. * p<0.05 (using the paired t-test). ** p<0.01 (using the Student's t-test).

4.3 Statistical Analysis

The analysis of effectiveness was performed for the subjects who completed the planned study schedule and test items, excluding those falling under any of the criteria for exclusion from analysis: 1) the subjects who consumed the test product too infrequently (less than 80%); 2) the subjects who considerably impaired the reliability of test results by missing records, violating restriction requirements, etc.; 3) the subjects who came under the subject exclusion criteria and were found as such after enrolment; and 4) the subjects who had clear reasons for exclusion from analysis.

Values were expressed in the form of mean ± standard deviation. Intragroup comparisons were made using a paired t-test, and intergroup comparisons were made using a Student's t-test. Statistical significance was defined as two-tailed P < 5%. Data were analysed using IBM SPSS Statistics ver. 22 (IBM Japan, Ltd., Tokyo).

Results

Of the 70 subjects who voluntarily expressed the intention to participate and supplied written consent, 7 declined to participate, and 19 were disqualified by the physician based on the results of a background survey, blood tests, and urinalysis, leaving 44 subjects in the study. These subjects were allocated randomly to the GME group (22 subjects) and the placebo group (22 subjects), and the study was started using these 2 groups. Excluding the 1 subject who voluntarily withdrew from the study midway, 43 subjects completed the study. Excluding the 2 subjects who fell under the criteria for exclusion from analysis, 41 subjects were analysed. The results concerning the background factors in each group are shown in Table 2. None of the items showed a significant difference between the GME group and the placebo group.

Table 3 shows the MED in Week 0 and Week 8, as well as the changes between these points, for the GME group and the placebo group. During the study period, MED in the GME group increased significantly from 29.2 ± 5.9 mJ/cm^2 to 30.7 ± 5.6 mJ/cm^2 (p < 0.05), showing a 5.1% increase in MED. On the other hand, MED in the placebo group showed a non-significant decreasing tendency (p = 0.10), resulting in a 5.0% decrease in MED. The changes in MED during the 8 weeks were 1.5 ± 3.0 mJ/cm^2, as compared with -1.5 ± 4.0 mJ/cm^2 in the placebo group. The difference between the groups was significant (p < 0.01).

With respect to safety, no side effects attributable to the test product were observed throughout the study period. For all other observation items, the principal investigator concluded that no safety problems occurred in this study.

Discussion

This study examined the effect of 8-week oral consumption of GME on MED in healthy Japanese males. The results showed a significant increase in MED among the subjects who consumed 250 mg/day of GME. MED shows seasonal changes, increasing in winter and decreasing in summer (Nakayama, 1977). The non-significant decrease in MED observed in the placebo group at the end of the study can be explained by this seasonal change (Table 3). In contrast, MED in the GME group increased significantly during the 8 weeks (Table 3), indicating that the oral consumption of GME improved the resistance to ultraviolet radiation. In an animal study, it was reported that the intravenous injection of bovine SOD to mice suppressed the formation of sunburn cells after ultraviolet irradiation (Danno et al., 1984).

The increase in antioxidant activity in blood enhances the tolerance to sunburn and suppresses the development of sunburn due to ultraviolet irradiation. The oral consumption of GME has been shown to raise antioxidant enzyme activities in the body in human and animal studies (Vouldoukis et al., 2004; Cloarec et al., 2007). It is, therefore, considered that the improvement of the antioxidant enzyme activities in the body resulting from the oral consumption of GME is involved in the increase in MED.

A similar study conducted in France showed that MED increased by 1.7-8.8% from the baseline in the group treated with 500 mg/day of GME, as compared with 1.0-1.2% in the placebo group (Mac-Mary et al., 2007). This article, however, merely reported that the MED in the GME group increased considerably as compared with the level in the placebo group, without stating

whether or not the increase was significant. On the other hand, our study demonstrated a similar extent of increase in MED using 250-mg doses, which were only half as large, and a significant difference in MED was confirmed in comparison with the placebo group (Table 3). Our results revealed that 250 mg/day of GME consumed by the Japanese was as effective in boosting MED as 500 mg/day. The daily dose of 500 mg has typically been used in the human clinical studies of GME that demonstrated its effectiveness in, for example, the prevention of atherosclerosis and suppression of DNA damage from hyperbaric oxygen therapy (Muth et al., 2004; Cloarec et al., 2007). In view of the results of this study, it is considered probable that these preventive effects can be obtained from the consumption of GME in a daily dose of 250 mg in the case of the Japanese.

This study demonstrated that the 8-week oral consumption of GME increased the minimal erythema dose (MED) of ultraviolet irradiation and improved the resistance of the skin against ultraviolet irradiation in healthy Japanese subjects.

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Abbreviations

GME, Gliadin-combined SOD-rich Melon Extract; MED, Minimal Erythema Dose; SOD, superoxide dismutase