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ORIGINAL ARTICLE

Effect of Soy Phosphatidylserine Supplemented Diet on Skin Wrinkle and Moisture *in Vivo* and Clinical Trial

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Abstract Effect of supplementation of phosphatidylserine (PS), enzymatically synthesized from soy lecithin, on skin moisture and wrinkle in animal and human was investigated. Skin moisture content of hairless mice was significantly decreased and skin thickness and wrinkle were significantly increased by UV irradiation, whereas PS-supplemented groups showed reduced wrinkle depth and much less wrinkle area unlike UV control (UV/Con) group. The collagen content in PS-supplemented groups increased compared with that in UV/Con group. A placebo-controlled double-blind intake study of soft capsules containing PS (300 mg/day) was performed on 63 subjects who had normal healthy skin for 12 weeks. Dermatologist's visual assessment and image analysis of replicas showed that wrinkle near eye was improved by PS-supplementation. Upon measurement of moisture content in the skin, PS supplementation increased the moisture content in the skin. These findings showed that PS supplementation was effective in moisturizing and improving wrinkle of the skin in both animal and human; thus, PS could be used as an effective skin food ingredient.

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Keywords anti-wrinkle · moisturizing effect · phosphatidylserine · skin barrier function · supplementation

Introduction

Skin aging can be caused by either intrinsic factor or extrinsic factor. Exposure to UV light, one of the extrinsic factors, has been known to be the major cause of physiological and morphological changes in skin aging (Meinhard et al., 2001). UV light can generate reactive oxygen species (ROS) in cells and tissues through various biological processes including enzymatic reactions after deep infiltration into epidermis and dermis, and cause direct effect on the skin. Overproduction of ROS may lead to various alterations including genetic mutation, inflammation, apoptosis, cancer, and skin aging (Li et al., 1996). ROS can also ruin normal skin function by oxidizing the lipid on the cell membrane, followed by the cell membrane damage. Moreover, failure of recovery from persistent oxidative status by repeating breakage of the anti-oxidation defence system on the skin can make the skin rough and dull, which may lead to wrinkles (Huang et al., 1997; Park, 2003). Collagenase is also one of the main causes inducing alteration and destruction of collagen fiber. ROS generated in the body can decrease elasticity of the skin and facilitate wrinkle formation by activating collagenases including matrix matalloproteinases (MMPs) and inhibiting biosynthesis of collagen (Seo et al., 2001). Therefore, it is crucial to identify anti-oxidant materials to remove ROS or compounds to inhibit collagenase activity and promote the biosynthesis of collagen for improving skin aging and wrinkle.

Though topical application of anti-oxidant compounds onto the skin is widely used to prevent skin aging, several problems exist in this method, including instability in the atmosphere and limited permeability into the skin. More interest are focused on production

of nourishing food for long-term preventive effect and understanding the relationship between specific nutrients and the skin to overcome these problems. One of the most actively investigated fields is the study to reveal the scientific action mode of most representative anti-oxidant foods including green tea, flavonoids, carotenoids, and vitamin in prevention of skin aging (Choi, 2002; Im et al.,; Lee et al., 2003).

Phosphatidylserine (PS) is a membrane phospholipid component that is ubiquitously present in prokaryotic and eukaryotic cell membranes (Vance and Steenbergen, 2005). In mammalian cells, cytosolic leaflet is composed of 20–30% PS and negative charge of PS has been shown to direct positively charged protein to the endocytic pathway (Yeung et al., 2008). Moreover, it has been demonstrated that PS, as a cofactor, activates several key signalling proteins, such as protein kinase C (Verdagure et al., 1999), Raf1 kinase (Improta-Brears et al., 1999), and neutral sphingomyelinase (Tomiuk et al., 2000). PS has been shown to interact with the peroxisome proliferator-activated receptor (PPAR) α protein, suggesting that PS could be a novel ligand of PPAR α (Van Veldhoven et al., 2000). PPARs are members of the nuclear hormone receptor super-family that have been reported to play an important role in maintaining epidermal homeostasis (Kuenzli and Saurat, 2003). Because activators of PPAR α decrease UV-induced cutaneous inflammation (Kippenberger et al., 2001), PS may also decrease UV-induced inflammation and retard photoaging.

Recently, the inhibitory effect of PS against MMP-1 expression and type-I collagen decreased by UV exposure in topical application has been reported (Cho et al., 2008). However, whether intake of PS can induce any inhibitory effect on skin aging and wrinkle formation still remains to be investigated. Therefore, the present study reports the effect of oral supplementation of PS in hairless mice on the improvement of the wrinkle of skin and skin moisture. As a human clinical test, we also investigated the effect of oral supplementation of PS on skin moisture content and wrinkle using microscopic three-dimensional skin surface analysis system in addition to diagnosis by dermatologists.

Materials and Methods

PS. Food grade PS was produced from soy lecithin by enzymatic transphosphatidylation, and contained PS 54.5%, phosphatidylcholine (PC) 3.0%, phosphatidylethanolamine (PE) 13.5%, phosphatidylinositol (PI) 5.0%, phosphatidic acid (PA) 10.0%, and others (neutral lipid, glycolipid, water etc.) 14.0%. PS was provided by Doosan Corporation Glonet BG (Korea) and PS was kept in the freezer (−60°C) and was used throughout the animal study.

For human clinical test, soft capsule containing oil type PS (Doosan phosphatidylserine 20L, PS 20%; Doosan Corporation Glonet) produced by dissolving enzymatically synthesized PS in medium chain triglyceride (MCT) was provided by Doosan Corporation Glonet BG (Korea). Each active capsule contained 150 mg pure PS. The placebo was a capsule containing MCT with the same appearance as the test supplement.

Animals and test material. Six- to seven-week old female hairless mice (Skh; HR-1) weighing 20–25 g were used in the study. Animals had been stabilized for a week before the study started. Food and drinking water were provided ad libitum and in controlled temperature (24±2°C), humidity (50±10%), and light cycle (12 h light and 12 h dark). Animals were divided into five groups of 6 mice each and treated for 10 weeks. The study groups consisted of normal control (Nor/Con) group, UV/Con group, low dose PS (12.5 mg/kg, LPS) group with UV treatment, medium dose PS (25 mg/kg, MPS) group with UV treatment, and high dose PS (50 mg/kg, HPS) group with UV treatment (KFRI-M-11007).

Exposure to UV light in animal test. To generate UV light, which is the most similar to that in the sunlight, UVB lamp (UB800, Waldmann Lichttechnik GmbH, Germany) was used. Animals, except those assigned to Nor/Con group were exposed to the UVB light three times per week on their back for 10 weeks at 60 mJ/cm^2 as minimal erythromal dose (MED) during the 1st week, 2 MED (120 mJ/cm^2) during the 2nd week, 3 MED (180 mJ/cm^2) during the 3rd week, and 4 MED (240 mJ/cm^2) from 4th week to the end of the study.

Observation of the clinical signs and body weight in animal test. Animals were observed once a day throughout the stabilization and treatment periods to find any clinical signs or death. The body weight of animals was measured once before the study started and once every two weeks during the study period.

Skin moisture and thickness in animal test. The skin moisture contents on the backs of animals were measured using Corneometer CM825 (Courage & Khazaka Electronics). The skin thickness on the backs of animals was measured using micrometer (Mitutoyo Co., Japan). The micrometer can determine as thin as 0.01 mm and is equipped with a function to put constant pressure on the thickness so that it was possible to measure the skin thickness under the same pressure.

Visual observation and replica image analysis in animal test. Visual observation was conducted at weeks 0, 5, and 10, and skin replica was collected at week 10 to evaluate the change in wrinkle formation. Skin replica was collected after drying the silicon polymer (Replica kit, Silflo, Flexico Ltd., UK) applied on the back of hairless mice. Collected skin replicas were analyzed using Visioline VL650 (Courage & Khazaka Electronics) to measure the gross surface area made by the wrinkles via quantification of the numbers and depths of the wrinkles calculated from the size of the shadow made by the wrinkles under the light at certain angle.

Histochemical assessment of the skin tissues in animal test. The tissue was prepared from the back skin (1 × 1 cm) of the sacrificed animal, placed evenly on the filter paper, fixed in 10% neutral formalin, and embedded in paraffin. The paraffin-embedded tissues were observed after Hematoxylin & Eosin (H&E) staining and Masson-Trichrome staining of 4-μm thick slice. The thickness of epidermis was determined by measuring the length from granular layer to basal layer of the epidermis on the H&E stained

tissue observed under the microscope with magnification of ×40. Collagen content was determined by measuring the area, shown as blue color on the Masson-Trichrome stained tissue observed under the microscope with magnification of ×40. The calculations were conducted by image analysing program, Optimas 6.5 (Optimas Co, USA).

Subjects for human clinical test. Eighty applicants of 40–60 year-old healthy normal individuals were recruited from Gyeonggyi, Korea. Ten people withdrew their consent or were excluded by exclusion rule. For example, people who had been treated with drugs such as oral or topical drugs or cosmetics having anti-wrinkle or moisturizing activity were excluded. After the study started, seven subjects withdrew from the study due to personal reasons. Therefore, 63 subjects were finally evaluated. This study was performed in conformity to the Helsinki declaration. Before execution of the study, physicians fully explained the purpose of this study to the subjects, and written consents were obtained (EL-111018361X110).

All subjects satisfied the inclusive criteria with periorbital wrinkles (global photodamage score 2–6) (Chung et al., 2001), confirmed by a dermatologist's medical interview and physical examination (Paepe et al., 2000). None of the subjects had used topical anti-wrinkle agents to treat their periorbital wrinkles within 3 months prior to this study. None of the subjects had undergone wrinkle removal or peeling procedures within 6 months prior to the study. None of the subjects were pregnant or breastfeeding or had atopic dermatitis, allergic diathesis or hypersensitive skin. All subjects were informed by the investigators about the study objectives, outlines, test methods and possible adverse effects. Subjects completed their profile, case report form and questionnaire, and signed the informed consent statement. The present study was a placebo-controlled double blind study of 12-week intake of the supplement containing PS (300 mg/day).

Anti-wrinkle and skin moisturizing effect in human clinical test. Clinical evaluations for anti-wrinkle effect were made at week 0 (baseline), 6, and 12. Visual assessment of baseline photographs of dermatologists, obtained from each subject, and image analysis of replicas using a Visiometer (Skin-Visiometer SV 600; Courage & Khazaka) were used to analyze changes in skin wrinkles (Chung et al., 2001). Periorbital wrinkles of subjects were evaluated in a double-blind test by dermatologists at weeks 0, 6, and 12 based on a global photodamage score of 0, none; 1, nonemild; 2, mild; 3, mildmoderate; 4, moderate, 5, moderatesevere; 6, severe; and 7, very severe during PS supplementation. Replicas of wrinkles in the left and right periorbital areas were acquired. Light intensity was analyzed with the Lambert-Beer law, and the degree of skin wrinkle improvement was calculated.

$$I_{ex}=I_{in}\ e^{-kd}$$

where I_{ex} is the transmitted light intensity, I_{in} is the unattenuated light intensity, k is the Napierian absorption coefficient of the medium, and d is the thickness of the medium. Evaluations were performed in the same location with the same lighting at each visit. Parameters used in the assessment of skin with the Visiometer SV 600 were as follows: R1, distance between the highest mountain and the lowest value; R2, biggest value of the five maximum distances; R3, average of the five maximum distances; R4, smoothness depth; R5, arithmetic average roughness. Clinical evaluations for skin moisturizing effect of the supplement containing PS were made at weeks 0 (baseline), 6, and 12. Corneometer CM825 (Courage & Khazaka Electronics) was used to analyze changes in skin conductance.

Statistical analysis. All measurement data were shown as mean and standard deviation. Statistical significance of all results in animal and human clinical test was analyzed by Student's t-test. The statistical data analysis was performed using SPSS statistical software version 10.0 (SPSS Inc., USA).

Results

Observation of the clinical signs and body weight in animal test. Dead case and specific clinical symptom by PS supplementation were not found in all study groups including Nor/Con group during the 10-week study period. In addition, significant differences in the body weight (24.0±2.4 g) were not found among the study groups prior to induction of wrinkle formation by UV exposure (Fig. 1). The body weight change during PS supplementation period did not show any significant difference among the study groups, and the body weight measured at the end of the study was 27.6±0.7 g. There was no significant difference in food or drinking water consumption among the study groups throughout the study period.

Change in skin thickness and moisture in animal test. In the case of skin aging by UV light, the skin becomes thick due to increased epidermal growth to protect dermal layer, and the skin moisture content decreases by the stratum corneum (Yoshinore et al., 1995). Therefore, thickened skin resulting from skin aging by UV light indicates skin damage by aging process (Gail, 2002). The skin thickness of the UV/Con group increased to 1.55±0.16

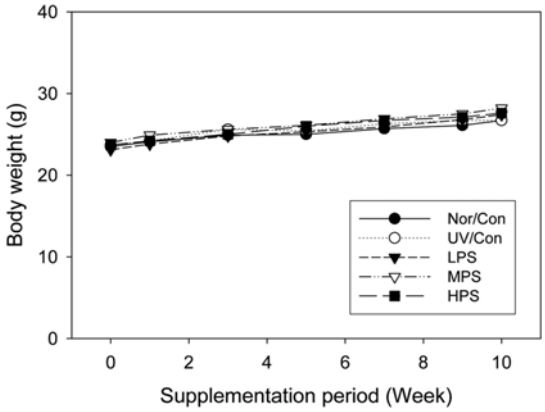

Fig. 1 Changes in body weights of hairless mice orally administrated with PS.

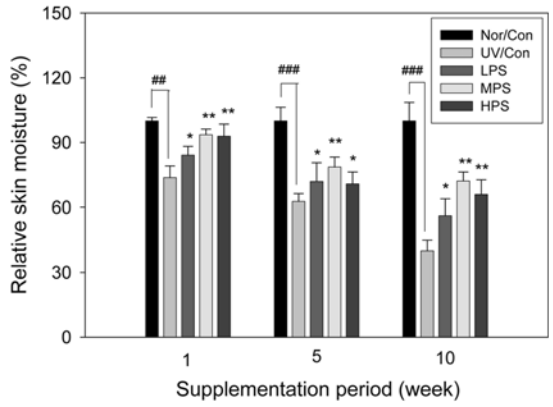

Fig. 2 Effect of PS on the relative skin moisture of hairless mice exposed to UV irradiation with different administration period. Each value was expressed as the mean ± SD of triplicate experiments. ## and ### represent significant difference between Nor/Con and UV/Con, p <0.01 and p <0.001, respectively. * and ** represent significant difference compared to UV/Con, p <0.05 and p <0.01, respectively.

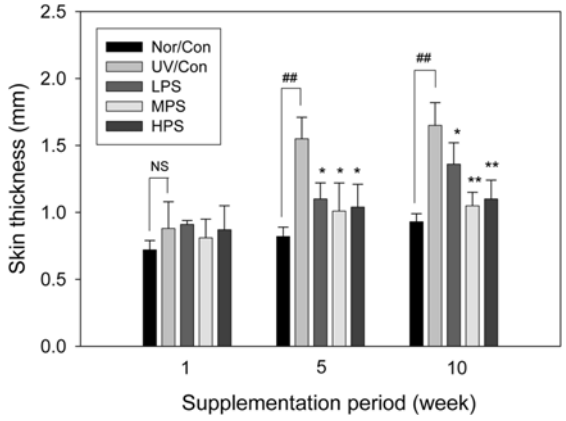

Fig. 3 Effect of PS on the skin thickness of hairless mice exposed to UV irradiation with different administration period. Each value was expressed as the mean ± SD of triplicate experiments. ## represents significant difference between Nor/Con and UV/Con, p <0.01 * and ** represent significant difference compared to UV/Con, p <0.05 and p <0.01, respectively. NS: no significance between two samples.

and 1.65±0.17 mm at weeks 5 and 10, respectively, whereas those of the Nor/Con group were 0.82±0.07 and 0.93±0.06 mm, respectively. On the other hand, the skin thickness of PS-supplemented groups were 1.01±0.21~1.10±0.12 and 1.05±0.10~1.36±0.16 mm at weeks 5 and 10, respectively, showing a significant decrease compared with that of UV/Con group (Fig. 2). Whereas the skin moisture of the UV/Con group decreased dramatically to 62.7±3.6 and 40.0±5.0% of the Nor/Con group at weeks 5 and 10, respectively, those of PS-supplemented groups under UV radiated condition were improved to 70.8±5.5~78.6±4.6 and 56.3±7.6~72.1±4.2% of the Nor/Con group at weeks 5 and 10, respectively, a significant increase compared with the UV/Con group (Fig. 3).

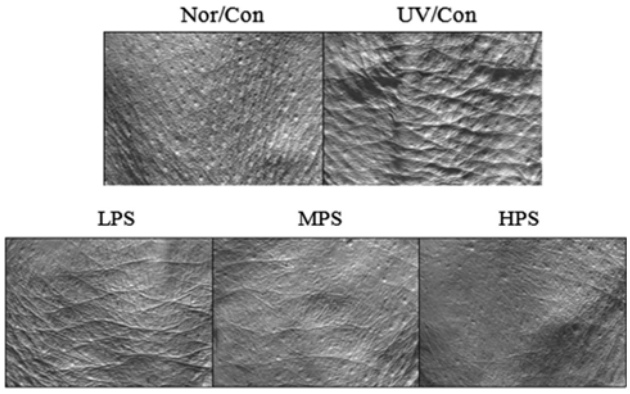

Fig. 4 Features of dorsal skin of hairless mice orally administrated with PS at the end of the administration period.

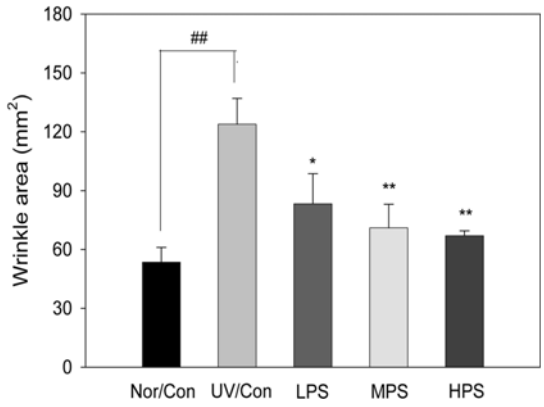

Fig. 5 Effect of PS on the wrinkle area of hairless mice exposed to UV irradiation. Each value was expressed as the mean ± SD of triplicate experiments. ## represents significant difference between Nor/Con and UV/Con, p <0.01 * and ** represent significant difference compared to UV/Con, p <0.05 and p <0.01, respectively.

Replica image analysis in animal test. The difference in the extent of wrinkle formation between Nor/Con group and UV/Con group was obvious, and the improvement of the wrinkle in the PS-supplemented groups was identifiable by visual observation. The difference was quantified by analyzing the skin replicas using Skin Visioline. Although higher increases in depth and number of wrinkles were observed in UV/Con group compared with Nor/Con group, PS-supplemented groups showed improvement in wrinkles compared with UV/Con group (Fig. 4). The improvement becomes more distinct in PS in a dose-dependent manner. In the wrinkle area analysis, whereas the wrinkle area of UV/Con group increased to 123.8±12.6 mm^2 compared with that of Nor/Con group showing 53.5±7.5 mm^2, those of PS-supplemented groups under UV irradiated condition were observed to be 67.0±2.5~83.3±15.3 mm^2, showing great improvement in dose-dependent manner (Fig. 5).

Histochemical observation of the skin tissue in animal test. Generally, the histochemical changes by long-term exposure to

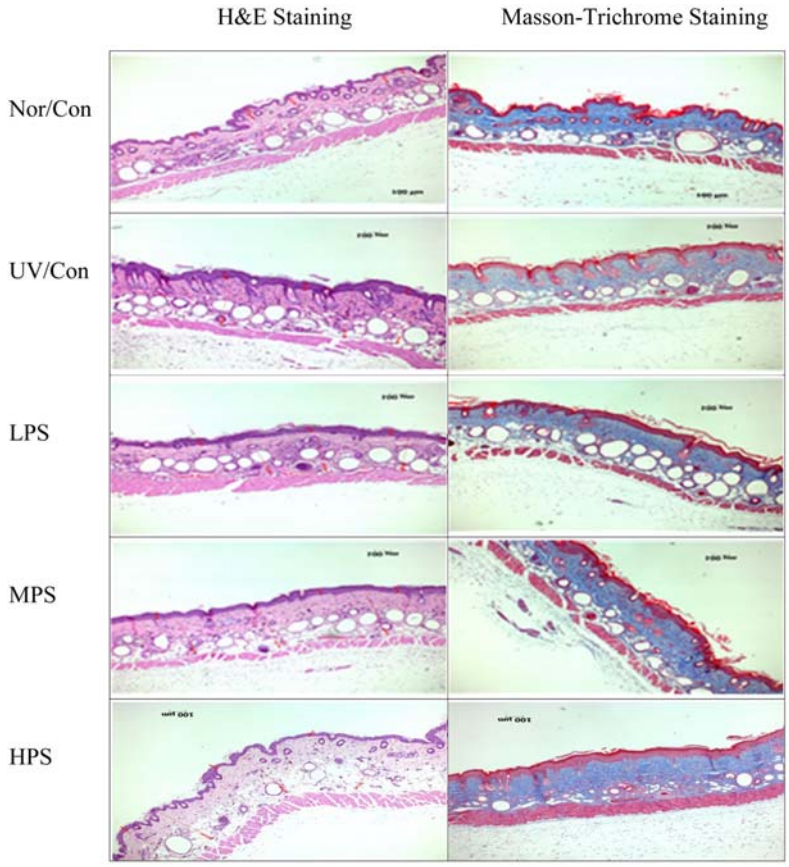

Fig. 6 Histological section of dorsal skin of hairless mice exposed to UV irradiation. At the end of 10-week oral administration of PS, skin biopsies were obtained from central dorsal skin to measure epidermal thickness. H&E and Masson-Trichrome staining (×200).

UV light include 2~3 times increase in thickness of epidermis, increase of prickle cells, overproduction of keratinocyte in epidermis, and structural alteration of collagen and elastin fiber, and elastosis by increase of elastic fiber in dermis. The histochemical analysis using back skin of animals stained with H&E and Masson-Trichrome revealed that the thickness of epidermis and dermis in UV/Con group was increased compared with that of Nor/Con group, and the thickness of epidermis and dermis in PS-supplemented groups was greatly decreased in dose-dependent manner compared with that of UV/Con group (Fig. 6). Moreover, whereas alteration of collagen distribution in dermis was observed in UV/Con group, PS-supplemented groups showed great decrease in tissue damage, suggesting that PS may have protective effect against tissue damages in epidermis, dermis, and subcutaneous fat layer induced by UV light.

In the analysis of epidermal thickness based on the H&E stained tissue section, the epidermal thickness of UV/Con group was 65.3±3.9 μm, which was greatly increased compared with that of Nor/Con group of 28.3±0.8 μm. The epidermal thickness of PS-supplemented groups ranged from 35.2±2.1 to 50.0±2.1 μm, which was decreased in a dose-dependent manner, as compared with that of UV/Con group (Fig. 7).

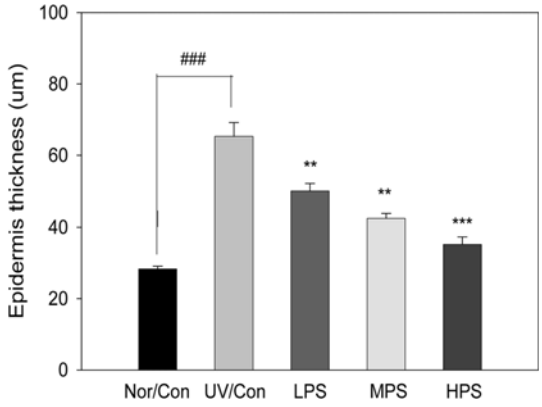

Fig. 7 Effect of PS on the epidermal thickness of hairless mice exposed to the chronic UV irradiation. At the end of 10-week oral administration of PS, skin biopsies were obtained from central dorsal skin to measure epidermal thickness. Each value was expressed as the mean ± SD of triplicate experiments. ### represents significant difference between Nor/Con and UV/Con, $p < 0.001$. ** and *** represent significant difference compared to UV/Con, $p < 0.01$ and $p < 0.001$, respectively.

When collagen content in the dermis was measured based on the Masson-Trichrome stained tissue section, the collagen content

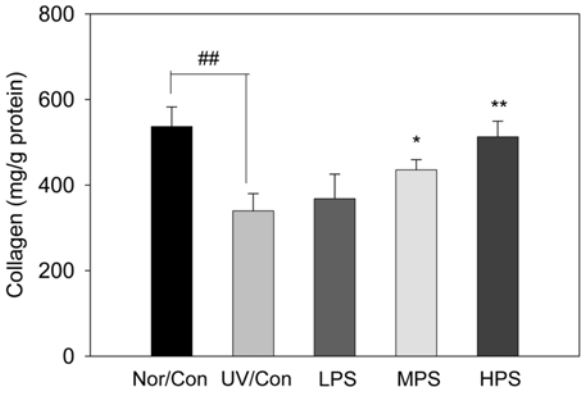

Fig. 8 Effect of PS on the collagen content of hairless mice exposed to the chronic UV irradiation. At the end of 10-week oral administration of PS, skin biopsies were obtained from central dorsal skin to measure epidermal thickness. Each value was expressed as the mean ± SD of triplicate experiments. ## represents significant difference between Nor/Con and UV/Con, p <0.01. NS; no significance between two samples.

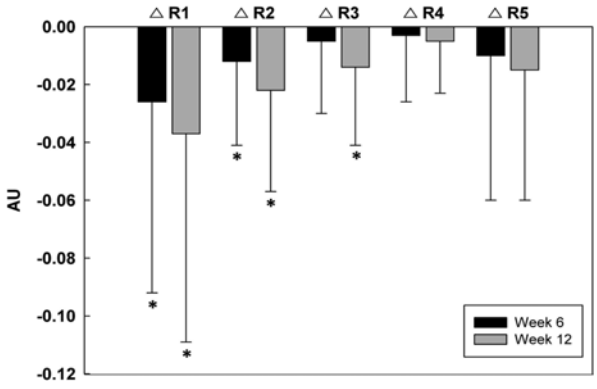

Fig. 9 Changes in wrinkles in PS-supplemented group in clinical study after 6- and 12-week supplementation of PS. R1, distance between the highest mountain and the lowest value; R2, biggest value of those five maximum distances; R3, average of the five maximum distances; R4, smoothness depth; R5, arithmetic average roughness. * represents significant difference between placebo and PS-supplemented group, p <0.05.

of UV/Con group was greatly decreased to 339.5±40.4 mg/g protein compared with that of Nor/Con group, 536.7±45.9 mg/g protein. The collagen contents of PS-supplemented groups ranged from 368.2±57.2 to 512.7±36.4 mg/g protein, which increased in a dose-dependent manner, as compared with that of UV/Con group (Fig. 8).

Anti-wrinkle effect in human clinical test. After 12 weeks of clinical study, the average photodamage score of applicants improved in the PS supplemented group compared with the placebo group (–0.32±0.48 vs –0.03±0.18, respectively, p=0.001) (Table 1).

A replica from the right and left periorbital areas was taken at weeks 0 (baseline), 6, and 12, and analyzed based on five parameters using the Visiometer and dedicated software. Parameters were compared at weeks 6 and 12 for PS-supplemented group in clinical study. Visiometer R-values (skin roughness values) decrease as wrinkles diminish (Fig. 9). At week 12, a statistically significant decrease in the skin roughness values, which are the arbitrary unit related to roughness in wrinkle formation was observed in PS-supplemented group. The improvement in R1, R2, and R3 was observed after 12 weeks (Fig. 9).

Therefore, based on the results of the clinical evaluations by dermatologist's visual assessment and image analysis using replicas and visiometers, we could conclude that 12 week intake of the supplement containing PS (300 mg/day) reduced the skin wrinkle in normal healthy people.

Skin moisturizing effect in human clinical test. Changes in the skin conductance in placebo and PS-supplemented group during the clinical study at weeks 6 and 12 are shown in Fig. 10A. At week 12, skin conductance value (Δ skin conductance) in PS-supplemented group was increased 8.44±9.47 as compared with that in placebo group (3.76±4.25) (Fig. 10B). In addition, statistically significant increase in the skin conductance values related to skin moisture content was observed in PS-supplemented group (p<0.05).Therefore, based on the results of the clinical evaluations by Corneometer, we could conclude that 12-week intake of the supplement containing PS (300 mg/day) increased the skin moisture content in normal healthy people.

Discussion

Skin aging is a complex biological process that includes natural and extrinsic aging, and produces functional and structural alterations to the skin. The skin photoaging process displays the prominent alterations in the skin through stimulation of multiple signal transduction pathways, which lead to activations of transcription factors or target genes (Fisher and Voorhees, 1998). Aged skin is smooth, pale, and finely wrinkled, whereas, photoaged skin shows coarse, deep wrinkles and dyspigmentation. Excessive exposure of UV on skin leads to photoaging process associated with wrinkling, sagging, and laxity. UV is known to induce the expressions of

Table 1 Changes in the global photodamage score in placebo and PS-supplemented group in clinical study

Week	Placebo	D	PS-supplemented	D	p-value
0	3.47±1.02		3.290±1.04		
6	3.44±0.95	-0.03±0.18	3.19±1.14	-0.10±0.30	0.083
12	3.44±0.98	-0.03±0.18	2.97±1.14	-0.32±0.48	0.001

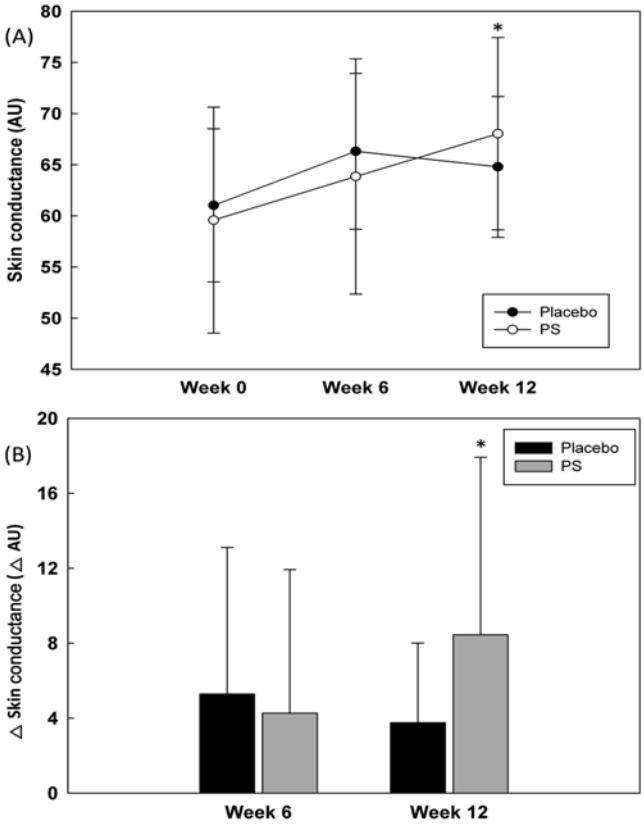

Fig. 10 Changes in the skin conductance in placebo and PS-supplemented group in clinical study after 6- and 12-week supplementation of PS. * represents significant difference between placebo and PS-supplemented group, $p < 0.05$.

MMP-1, -3, and -9, which regulate the synthesis and degradation of the extracellular matrix (ECM), in the human epidermal keratinocyte and dermal fibroblasts (Fisher et al., 1998). MMP expression is increased by various stimuli, including UV, oxidative stress, and inflammatory cytokines, and excessive MMP expression initiates ECM collagen degradation by cleaving fibrillar collagen at a single cleavage site. Once collagen is cleaved by MMP-1, further degradation occurs by MMP-3 (stromelysin) and -9 (gelatinase) (Brennan et al., 2003).

The UV-mediated induction of MMP-1 is associated with increased expression of several transcription factors and reactive oxygen species (ROS). UV-induced ROS is implicated in activating several signal transduction pathways and have been shown to activate the mitogen-activated protein kinase (MAPK) signalling pathway (Assefa et al., 1997). Fisher and Voorhees (1998) reported that UV exposure activates growth factor receptors, which induce the activation of protein kinase cascades, such as the MAPK cascades. The downstream targets of MAPKs, c-Jun, and c-Fos, form the activator protein-1 (AP-1) transcription factors. Fini et al. (1988) reported that transcription of MMPs is mainly regulated by AP-1 regulatory element in their proximal promoter region. UV exposure has diverse effects on the production of cytokines, among them IL-8 and MCP-1 were predominantly increased (Kang et al., 2007).

Cho et al. (2008) reported that PS had a stimulatory effect on procollagen and an inhibitory effect on MMP-1 expression. They also found the inhibitory effects of PS on acute UV response and chronologic skin aging by topically applying it to the skin of young people before UV irradiation and to the skin of elderly people, and found that topical PS treatment could increase procollagen transcription in the upper dermis, and significantly decrease MMP-1 expression at both mRNA and protein levels under intrinsic-aged condition.

Alterations occurring inside the skin tissue can ultimately lead to wrinkle formation by damaging normal skin function as a barrier to control absorption of foreign materials, moisture evaporation, thickening of the skin, and altering the structure of dermis (Haratake et al., 1997). Several studies using acute skin barrier damage model have demonstrated that recovery process occurring in damaged skin barrier involves with various signalling pathways, which induce supplement and new biosynthesis of lipid in a dynamic manner. Most representative signs of the recovery include improvement in transepidermal water loss (TEWL) and skin thickness (Davis et al., 1996). Chung et al. (2006) reported that promoting differentiation of keratinocytes could strengthen the skin barrier through increase of ceramide content and decrease of TEWL. Several studies reported that skin function and structure can be improved with supplementation of food nutrients essential for skin metabolism (Mac-Mary et al., 2006; Guillou et al., 2011). Change in the skin structure is a result of loss in barrier integrity, increased TEWL, and diminished skin hydration. De Spirt et al. (2012) also reported that intake of a fruit- and vegetable-based concentrate increased microcirculation of the skin at 12 weeks of intervention and positively affected skin hydration, density, and thickness.

As human beings age, the epidermis begins to lose its ability to hold moisture, leading to the appearance of fine lines in the skin's surface. With deepening in the wrinkle, collagen and elastin break down, and the support structure of skin weakens. Thus wrinkle formation and skin moisture content are very closely related.

In the present study, attempt was made to evaluate anti-wrinkle and skin moisturizing effect of PS supplementation in UV-irradiated animal model. Whereas UV exposure dramatically increased wrinkle formation in visual observation and thickness of epidermis, PS supplementation significantly decreased visual wrinkle formation and epidermis thickening compared with UV/Con group, as well as increased skin moisture content significantly. The decrease of collagen content induced by UV exposure was improved by PS supplementation compared with UV/Con group, which indicates that PS supplementation could inhibit wrinkle formation by promoting collagen synthesis. It was also observed that PS supplementation could significantly improve the moisture content of skin drastically decreased by UV exposure compared with Nor/Con group. PS supplementation also could decrease the skin

thickening by UV exposure with significance, showing improvement of skin barrier damage.

We also investigated the anti-wrinkle and skin moisturizing effects of PS on chronologic skin aging by dietary supplementation of PS to healthy 40–60 year-old women. Result of human clinical test revealed that 12-week intake of the supplement containing PS (300 mg/day) showed decreased wrinkle formation and improvement in skin moisturizing effects in subjects who had normal skin. Dermatological diagnosis and instrumental analysis showed that the PS supplementation increased the moisture content in the skin and lessened the formation of wrinkle near the eye.

The results of the present study show that supplementation of PS results in skin protective effect by improving skin barrier function such as improving skin hydration, as well as preventing wrinkle and loss of elasticity resulting from structural damage in dermis caused by exposure to UV, in addition to normal healthy condition. These results suggest that PS may have dual effect on skin protection including improvement of dermal matrix, as well as improvement in epidermis and skin barrier function, and PS can be utilized as a potential functional food supplement for skin moisturization, skin function protection, and wrinkle improvement in the future.

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