Journal of Nutrition & Food Sciences
ISSN: 2155-9600

Tokudome et al., J Nutr Food Sci 2012, 2:3
DOI: 10.4172/2155-9600.1000137

Research Article Open Access

Influence of Oral Administration of Soybean Peptide on Water Content of the Stratum Corneum, Transepidermal Water Loss and Skin Viscoelasticity

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Abstract

Soybean Peptide (SP) increases collagen content in fibroblasts, but no previous studies have investigated the effects of SP on skin properties such as water content of the stratum corneum, Transepidermal Water Loss (TEWL) and skin viscoelasticity. Thus, we orally administered SP to ultraviolet (UV)-irradiated mice in order to investigate the effects on skin. Hairless mice were irradiated with a daily dose of 22.3 J/cm^2 of UVA and a 20% aqueous solution of SP or Collagen Peptide (CP) was administered via oral gavage to achieve a daily dose of 2 mg/g. UVA radiation and oral samples were administered for 25 consecutive days. Water content of the stratum corneum and TEWL were measured daily and skin viscoelasticity was measured every other week. Skin was collected and prepared as frozen sections after the conclusion of the study for use in histological observation with hematoxylin and eosin staining. Oral administration of SP and CP to UVA-irradiated mice resulted in no significant differences in body weight in any of the groups. Water content of the stratum corneum and TEWL improved over time in all groups. TEWL recovered more quickly in the SP group than the CP group. Skin viscoelasticity improved in all groups. The values in the SP group were almost identical to those in the normal group, which did not receive UVA irradiation or oral treatment. Histological observations revealed suppression in UVA-induced epidermal thickening in the SP group. The findings of this study indicate that oral administration of CP and SP improves the barrier function of skin and skin viscoelasticity in UV-irradiated mice.

Keywords: Soybean peptide; Stratum corneum; Water content; Transepidermal water loss; Skin viscoelasticity; Oral administration

Introduction

The skin consists of the epidermis and the dermis. From the inside out, the epidermis consists of the stratum basale, the stratum spinosum, the stratum granulosum and the stratum corneum. The stratum corneum of outermost layer of the epidermis acts as a barrier protecting against ultraviolet (UV) stimuli and foreign matter as well as preventing water loss through the skin. The dermis contains fibroblasts which produce growth factors, collagen and elastin. The intertwining of these substances and moisture-rich acidic mucopolysaccharides confers elasticity and rigidity to the skin.

UV radiation is electromagnetic radiation with a wavelength of 190 to 400 nm and can be classified as UVA, UVB and UVC. UVA, long-wavelength radiation over the range of 320 to 400 nm, penetrates deeply into the dermis, degenerating collagen and causing wrinkles and sagging. Even small doses of UV adversely affect the skin after long-term exposure. UV injures the stratum corneum, reducing its barrier functionality and causing the loss of water from the skin, resulting in skin dryness. Water content of the stratum corneum [1-3], Transepidermal Water Loss (TEWL) [4-7] and skin viscoelasticity [8,9] are commonly used as indexes to evaluate skin pathologies.

Peptides having a molecular weight of several thousand form when proteins undergo biological processes such as enzymatic breakdown or hydrolysis. Soybean peptide (SP) reduces blood cholesterol [10,11], increases muscle protein content [12] and increases collagen content in fibroblasts [13]. Orally administered collagen peptide (CP) improves the water content of the stratum corneum and normalizes TEWL in UVB-irradiated mice [14] and increases the water content of the stratum corneum [15] and skin viscoelasticity [16] in humans. However, no reports in the literature have investigated the effects of SP on skin characteristics such as water content of the stratum corneum, TEWL and skin viscoelasticity. We previously demonstrated that SP increases the collagen content of human fibroblasts [17]. Collagen is one component of the extracellular matrix in the dermis within the skin.

In this study, we administered SP to UV-irradiated hairless mice in an attempt to increase the collagen content of the skin and compared the results to those obtained with CP.

Materials and Methods

Study samples

The SP used was HI-NUTE AM (Fuji Oil Co. Ltd., Osaka, Japan) and the CP was extracted from fish (SCP-AS-L; Nippi Inc., Tokyo, Japan). Amino acid compositions were as described previously [13,17].

Reagents

Xylene and Weigert's iron hematoxylin solution were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Somnopentyl® was purchased from Kyoritsu Seiyaku Corporation (Tokyo, Japan). Optimal Cutting Temperature (OCT) compound was procured from Sakura Finetek Japan Co., Ltd. (Tokyo, Japan) and the

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Received February 28, 2012; **Accepted** April 16, 2012; **Published** April 23, 2012

Citation: Tokudome Y, Kage M, Hashimoto F (2012) Influence of Oral Administration of Soybean Peptide on Water Content of the Stratum Corneum, Transepidermal Water Loss and Skin Viscoelasticity. J Nutr Food Sci 2:137. doi:10.4172/2155-9600.1000137

agent for sample embedding was purchased from Matsunami Glass Ind., Ltd. (Osaka, Japan).

Animal experiments

Seven-week-old, male, HR-1, hairless mice (average body weight, 20 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). Mice were maintained in a light- (12-h light/dark cycle) and temperature-controlled (25 ± 2°C) barrier facility throughout the study. All animal experiments and maintenance were performed under conditions approved by the animal research committee of Josai University. Mice were randomly assigned to four groups: normal, control, SP and CP (3 mice per group). The experimental groups are shown in (Table 1). Animals in the groups receiving UVA irradiation were exposed to 22.3 J/cm^2 every day for 25 consecutive days. UVA was administered with a high-performance UV transilluminator (UVP, LLC, CA, US). For 25 consecutive days, 10 mL/kg distilled water was orally administered daily to the normal and control groups and the respective samples at 2 g/10 mL/kg were orally administered to the SP and CP groups. To measure skin viscoelasticity, a 10-fold dilution of Somnopentyl® was intraperitoneally administered at a dose of 0.01 mL/kg in order to anesthetize the animals. Water content of the stratum corneum and skin viscoelasticity were measured with a Cutometer MPA580 (Courage & Khazaka, Cologne, Germany) and TEWL was assessed using a VAPO SCAN AS-VT100 RS (Asahi Techno Lab. Ltd., Kanagawa, Japan). Measurements were taken for 8 seconds or, under reduced pressure (300 mb), for 4 seconds (Table 1).

Hematoxylin and eosin staining

Cryosections were prepared from tissue samples embedded in optimal cutting temperature (OCT) compound. Skin sections (10 μm) were stained with hematoxylin and eosin (HE) and analyzed for structural differences using light microscopy.

Data and statistical analysis

All results are expressed as means ± S.D. of three experiments. Statistical analysis was conducted using Dunnett's post-hoc test (using SAS software version 9.2).

Results

Body weight changes in UVA-irradiated mice after oral administration of SP and CP

Figure 1 shows the body weight changes in UVA-irradiated mice after oral administration of SP and CP. Although body weight in the UVA-irradiated groups was slightly lower, the differences among the groups were not significant.

Changes in water content of the stratum corneum in UVA-irradiated mice after oral administration of SP and CP

Figure 2 (A) shows the changes in water content of the stratum corneum following oral administration of SP and CP to UVA-irradiated mice. Figure 2 (B) shows the water content of the stratum corneum after 25 days. The water content of the stratum corneum in the normal group

Group	UVA irradiation	Application
Normal	None	Distilled water
Control	22.3 J/cm^2	Distilled water
SP	22.3 J/cm^2	2 mg/g SP (as 20% soln.)
CP	22.3 J/cm^2	2 mg/g CP (as 20% soln.)

Table 1: Experimental groups.

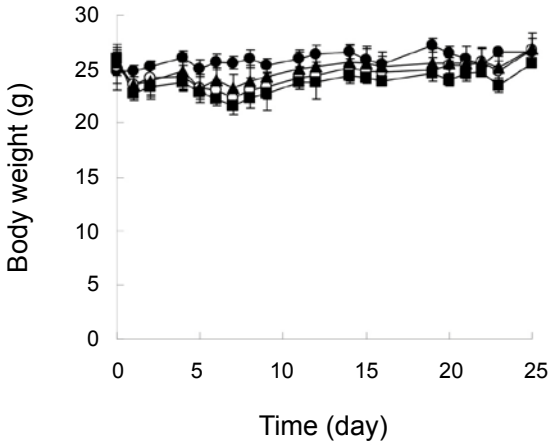

Figure 1: Body weight changes in UVA-irradiated mice after oral administration of SP and CP. Symbols and bars represent mean and standard deviation (n=3), respectively.
Closed circles, Normal (no UVA irradiation, distilled water); open circles, Control (22.3 J/cm^2 UVA, distilled water); closed squares, SP (22.3 J/cm^2 UVA, SP); closed triangles, CP (22.3 J/cm^2 UVA, CP).

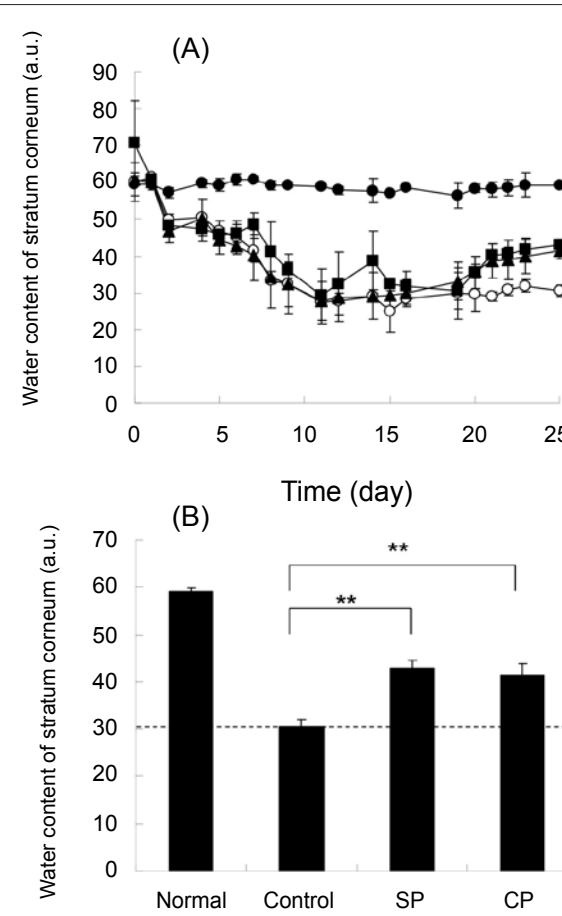

Figure 2: Water content of the stratum corneum of UVA-irradiated mice after oral administration of SP and CP over time (A), and after 25 days (B). Symbols and bars represent mean and standard deviation (n=3), respectively.
Closed circles, Normal (no UVA irradiation, distilled water); open circles, Control (22.3 J/cm2 UVA, distilled water); closed squares, SP (22.3 J/cm^2 UVA, SP); closed triangles, CP (22.3 J/cm^2 UVA, CP). *p < 0.05, **p < 0.01 vs. control group (Dunnett's post-hoc test).

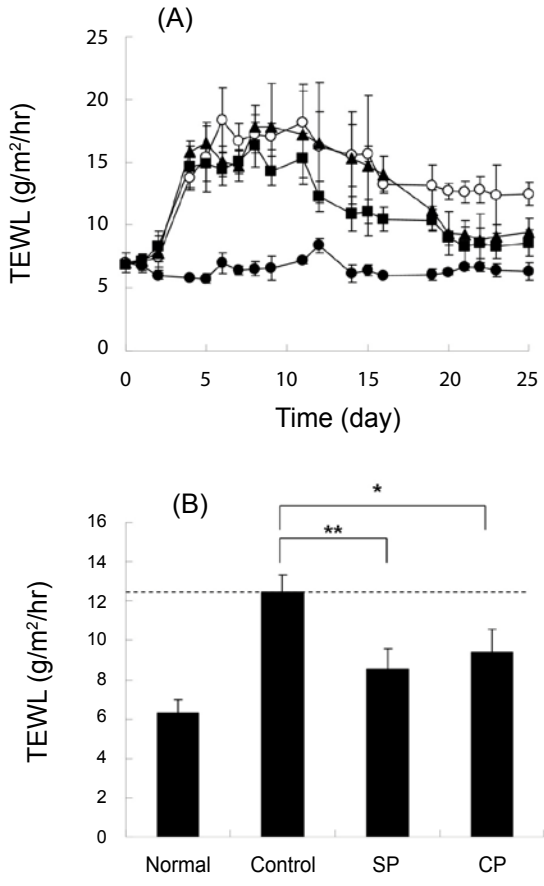

Figure 3: Transepidermal water loss (TEWL) in UVA-irradiated mice after oral administration of SP and CP over time (A), and after 25 days (B). Symbols and bars represent mean and standard deviation (n=3), respectively. Closed circles, Normal (no UVA irradiation, distilled water); open circles, Control (22.3 J/cm2 UVA, distilled water); closed squares, SP (22.3 J/cm^2 UVA, SP); closed triangles, CP (22.3 J/cm^2 UVA, CP). *p < 0.05, **p < 0.01 vs. control group (Dunnett's post-hoc test).

did not vary significantly throughout the study. Although the water content of the stratum corneum decreased following UVA irradiation, the water content increased after about 20 days in the groups receiving SP or CP relative to the group receiving distilled water. The water content of the stratum corneum was significantly higher in the SP and CP groups than in the distilled water group after 25 days.

Changes in TEWL in UVA-irradiated mice after oral administration of SP and CP

Figure 3 (A) shows the changes in TEWL following oral administration of SP and CP to UVA-irradiated mice. Figure 3 (B) shows TEWL after 25 days. TEWL in the normal group did not vary significantly throughout the study, but increased to approximately 15 g/m^2/h in all groups irradiated with UVA. TEWL decreased over time in the SP and CP groups. Recovery in the SP group began after about 10 days and proceeded more quickly than recovery in the CP group. TEWL was significantly lower in the SP and CP groups than in the distilled water group after 25 days.

Changes in skin viscoelasticity in UVA-irradiated mice after oral administration of SP and CP

Figure 4 (A) shows the changes in skin viscoelasticity following the oral administration of SP and CP to UVA-irradiated mice. Figure 4 (B) shows skin viscoelasticity after 21 days. Skin viscoelasticity in the normal group did not vary significantly throughout the study. Skin viscoelasticity was lower in all groups irradiated with UVA and began recovering after 14 days in the SP and CP groups. Skin viscoelasticity was significantly higher in the SP group than in the distilled water group after 25 days.

Skin sections in UVA-irradiated mice after oral administration of SP and CP

HE-stained skin sections from UVA-irradiated mice after oral administration of SP and CP are shown in Figure 5. Dotted red lines indicate the boundary between the epidermis and the dermis. UV-induced epidermal thickening was seen in the group irradiated with UVA and given distilled water in comparison with normal mice. Epidermal thickness was less pronounced in the SP and CP groups. Epidermal thickness was particularly suppressed in the SP group.

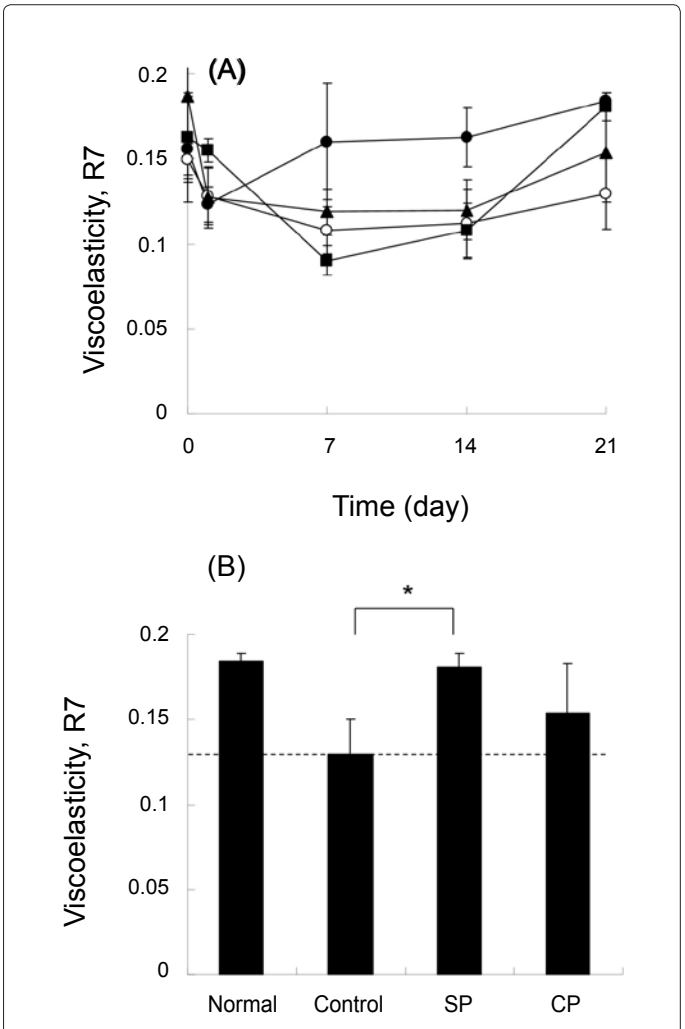

Figure 4: Viscoelasticity in UVA-irradiated mice after oral administration of SP and CP over time (A), and after 21 days (B). Symbols and bars represent mean and standard deviation (n=3), respectively. Closed circles, Normal (no UVA irradiation, distilled water); open circles, Control (22.3 J/cm^2 UVA, distilled water); closed squares, SP (22.3 J/cm^2 UVA, SP); closed triangles, CP (22.3 J/cm^2 UVA, CP). *p < 0.05 vs. control group (Dunnett's post-hoc test).

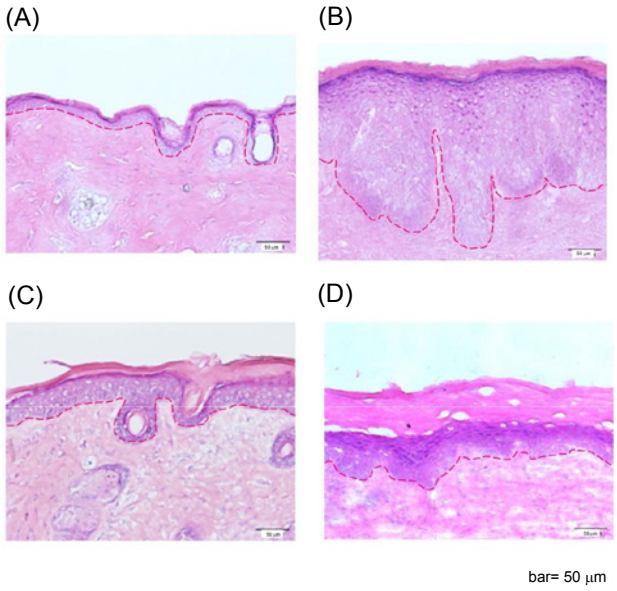

Figure 5: Hematoxylin-eosin (HE)-stained sections from UVA-irradiated mouse skin after oral administration of SP and CP at 25 days. (A) Normal (no UV irradiation, distilled water); (B) Control (22.3 J/cm^2 UVA, distilled water); (C) SP (22.3 J/cm^2 UVA, SP); (D) CP (22.3 J/cm^2 UVA, CP). Bars indicate 50 μm.

Discussion

We investigated the effects and benefits of SP on the skin and compared the results to those obtained with CP, the skin effects of which have already been characterized. Animals irradiated with a daily UVA dose of 22.3 J/cm^2 were used in the investigation [18]. UVA striking the skin penetrates to the dermis, where it is thought to cleave collagen fiber bundles, thereby inducing aging of the skin [19]. This hypothesis was behind the rationale for our selection of animal model.

The SP and CP used in the study contained large amounts of dipeptides and tripeptides having molecular weights of around 500. SP contains large quantities of aspartic acid and glutamic acid [17].

We orally administered SP and CP to UVA-irradiated mice in order to investigate the skin effects over time. The UV-irradiated animals weighed slightly less than the non-irradiated animals, but among UVA-irradiated animals, body weights did not differ among the distilled water, SP and CP groups. The effects of food intake are therefore thought to have been minimal.

Water content of the stratum corneum, TEWL and skin viscoelasticity were measured over time in order to investigate the effects of SP and CP on the skin. Water content of the stratum corneum began improving after 20 days in the SP and CP groups (Figure 2A). Water content of the stratum corneum was significantly higher in the SP and CP groups than the distilled water group at the end of the study. TEWL increased following UV irradiation, but decreased in the SP and CP groups (Figure 3A). A reduction in TEWL was observed in the SP group at 10 days after the start of administration, indicating that recovery was quicker than in the CP group. TEWL is often used as an indicator of barrier function. The findings suggest that SP effectively facilitates the repair of barrier destruction. Skin viscoelasticity was significantly higher in the SP group on day 21 of administration (Figure 4B). Notably, viscoelasticity was comparable to that in the non-irradiated animals. We used R7 as an indicator of viscoelasticity [20]. R7 indicates percent return at 0.1 seconds after the skin is subjected to reduced pressure at 300 mb for 4 seconds with a probe and is considered to be a highly reproducible measurement. It is very interesting to note that R7 increased significantly. We previously reported that SP treatment increases collagen production in fibroblasts and increases expression of the collagen 1A1 gene in cells [17]. SP may therefore increase the water content of the stratum corneum, lower TEWL and improve skin viscoelasticity by increasing collagen production in skin cells. We believe that SP administration may also affect the extracellular matrix and plan to investigate this in a future study. When skin morphology with HE-staining was observed, distinct epidermal thickening attributable to UVA irradiation was present in the control group. This UVA-induced epidermal thickening was suppressed in the SP and CP groups (Figure 5). Skin tissue in the SP group appeared to be morphologically similar to that in the non-irradiated group.

In conclusion, SP improved water content of the stratum corneum, TEWL and skin viscoelasticity in UVA-irradiated mice. The effects of SP were comparable to or greater than those associated with CP, the efficacy of which has previously been demonstrated.

Acknowledgement

The authors gratefully acknowledge the technical assistance of Ms. Chiaki Mori.

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