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Anti-wrinkling effects of "L-Skin Care" and molecular mechanisms on hairless mouse skin caused by chronic ultraviolet B irradiation.

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ABSTRACT

Background: Naturally occurring antioxidants were used to regulate the skin damage caused by ultraviolet (UV) radiation because several antioxidants have demonstrated that they can inhibit wrinkle formation through prevention of matrix metalloproteinases (MMPs) and/or increase of collagen synthesis.
Objective: We examined the effect of oral administration of the antioxidant mixture ("L-Skin Care") on UVB-induced wrinkle formation. In addition, we investigated the possible molecular mechanisms of photoprotection against UVB through inhibition of collagen–degrading MMP activity or through enhancing of procollagen synthesis in mouse dorsal skin.
Methods: Female SKH-1 hairless mice were orally administrated "L-Skin Care" (test group) or vehicle (control group) for 10 weeks with UVB irradiation by three times a week. The intensity of irradiation was gradually increased from 30 to 180 mJ/cm^2. Microtopographic and histological assessments of the dorsal skins were carried out at the end of 10 weeks to evaluate wrinkle formation. Western blot analysis and EMSA were also carried out to investigate the changes in the balance of collagen synthesis and collagen degradation.
Results: Our "L-Skin Care" significantly reduced UVB-induced wrinkle formation, accompanied by significant reduction of epidermal thickness, and UVB-induced hyperplasia, acanthosis and hyperkeratosis. Oral administration of "L-Skin Care" significantly prevented UVB-induced expressions of MMPs, mitogen-activated protein (MAP) kinases and activation of activator protein (AP)-1 transcriptional factor in addition to enhanced type I procollagen and transforming growth factor-β (TGF-β) expression.

Conclusion: Oral administration of "L-Skin Care" significantly inhibited wrinkle formation caused by chronic UVB irradiation through significant inhibition of UVB-induced MMP activity accompanied with enhancement of collagen synthesis.

Key words: "L-Skin Care", UV, wrinkle, MMPs, MAP kinase, AP-1

Modern nutritional science has been interested in the role of specific food supplements in reducing the risk of the skin disorders from ultraviolet (UV) irradiation. In recent years, naturally occurring compounds have gained attention as protective agents in food industry. The combination of vitamin C (ascorbic acid) and vitamin E (α-tocopherol) were reported to act synergistically to enhance free radical scavenging activity induced by UV irradiation in human skin . Pine bark extract (pycnogenol®), a concentrate of polyphenols and procyanidins, was recognized as a powerful antioxidant. It was also reported to show the protective effects against UV-induced skin damage in human clinical study. Antioxidant and anti-inflammatory effects, and promising natural treatments for scleroderma by evening primose oil have also been demonstrated. However the precise molecular mechanism of photoprotective effects of these antioxidants remains unclear.

Solar UV radiation is a major environmental hazard that leads to acute and chronic reactions in the human skin. Chronic exposures are the primary cause of premature aging of the skin so - called photoaging, which is characterized clinically by wrinkling, blotchy dyspigmentation, and rough skin textures. There is increasing evidence that UV radiation induces extensive generation of reactive oxygen species (ROS) in the skin . Previous studies in hairless mice indicated that UV-induced ROS were involved in wrinkle formation. Increased ROS generation can overwhelm the antioxidant defense mechanism, resulting in oxidative stress and oxidative photodamage of macromolecules and plasma membrane components in the skin. Wrinkle formation is thought to occur because of accumulated damage to the extracellular matrix that comprised the dermal connective tissue. Accumulating evidence from *in vitro* studies indicates that increased ROS generation activates the mitogen-activated protein (MAP) kinase signal transduction pathway, which induces the expression of activator protein-1 (AP-1) driven genes including matrix metalloproteinases (MMPs). AP-1 has also been reported to regulate type I procollagen gene expression negatively. This inhibition appears to result from the inhibition of procollagen transcription induced by transforming growth factor β 2(TGF-β2). UV-induced activation of MMPs and inhibition of procollagen synthesis finally leads to wrinkling, which has been implicated in photoaging.

In this study, we examined the protective effects on UVB-induced wrinkle formation in hairless mice by oral administration of "L-Skin Care". In addition,

we investigated the precise molecular mechanism of photoprotective effects of "L-Skin Care" against UVB-induced skin damages in mouse dorsal skin.

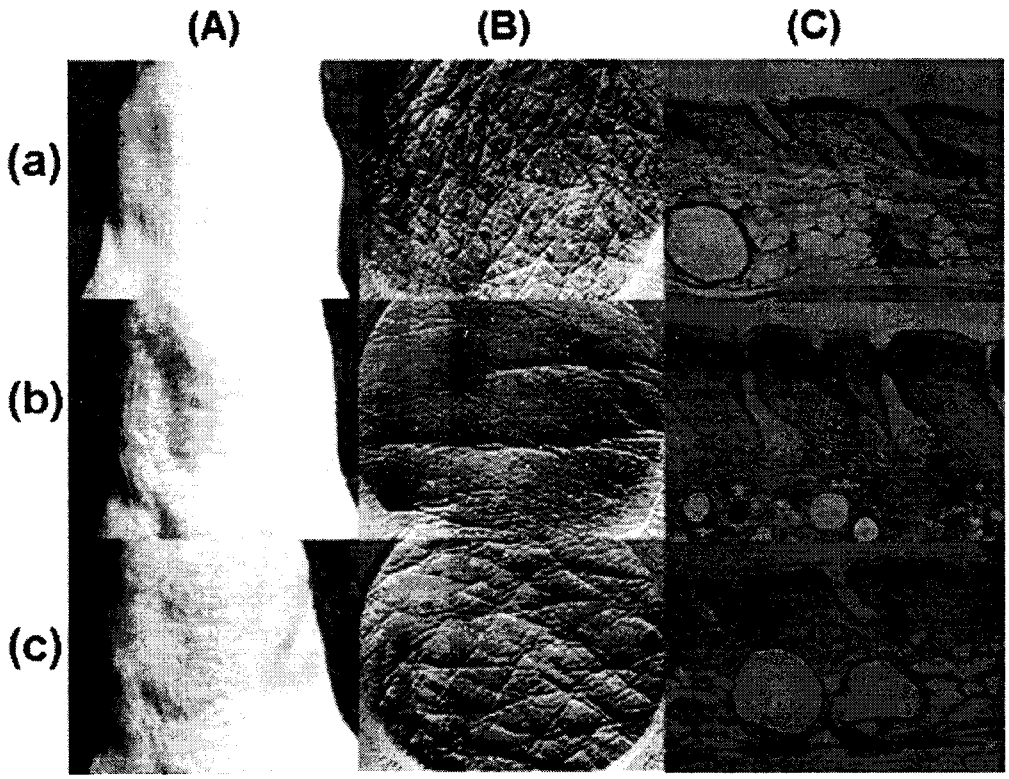

Fig. 1. Effects of oral administration of "L-Skin Care" on UV-induced wrinkle formation on the back of hairless mice skin at the end of week 10. Mice were divided into three groups and were orally administrated vehicle or "L-Skin Care" for 10 weeks. The mice of UV control group and "L-Skin Care" treated group were irradiated three times a week for 10 weeks. (a) Group 1 (non-UVB exposed); (b) group 2 (Vehicle + UVB); (c) group 3 ("L-Skin Care" + UVB). (A) Features of hairless mouse dorsal skins; (B) photographs of replica taken from the central dorsum of the mice; (C) histological sections of mouse dorsal skins. H&E staining. Magnification x 200.

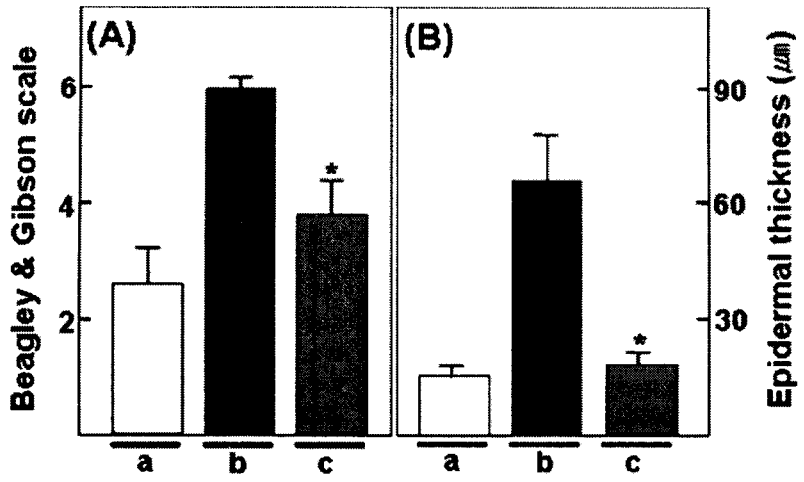

Fig. 2. Degree of wrinkling by Beagley and Gibson scale (A) and the skin epidermal thickness (B) of hairless mice dorsal skins at the end of week 10. a, Group 1 (non-UVB exposed); b, Group 2 (Vehicle + UVB); c, Group 3 ("L-Skin Care" + UVB). The values are mean±SD form 10 animals.

* Significantly different from UVB treated group ($p<0.05$)

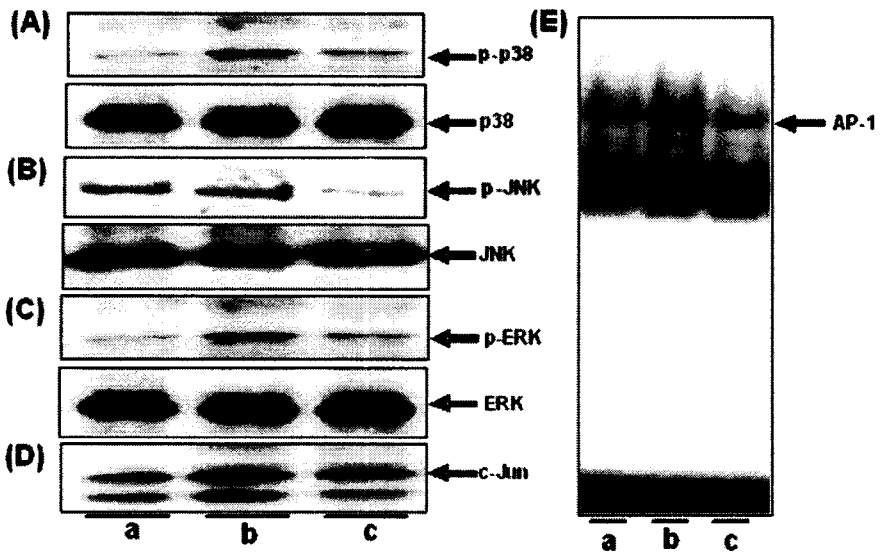

Fig. 3. Oral administration of "L-Skin Care" inhibits UVB irradiation-induced expressions of p38 MAPK (A), JNK (B), ERK (C), c-Jun (D), and the DNA binding activity of AP-1 in hairless mice skin. a, non-UVB exposed; b, Vehicle + UVB; c, "L-Skin Care"+UVB.

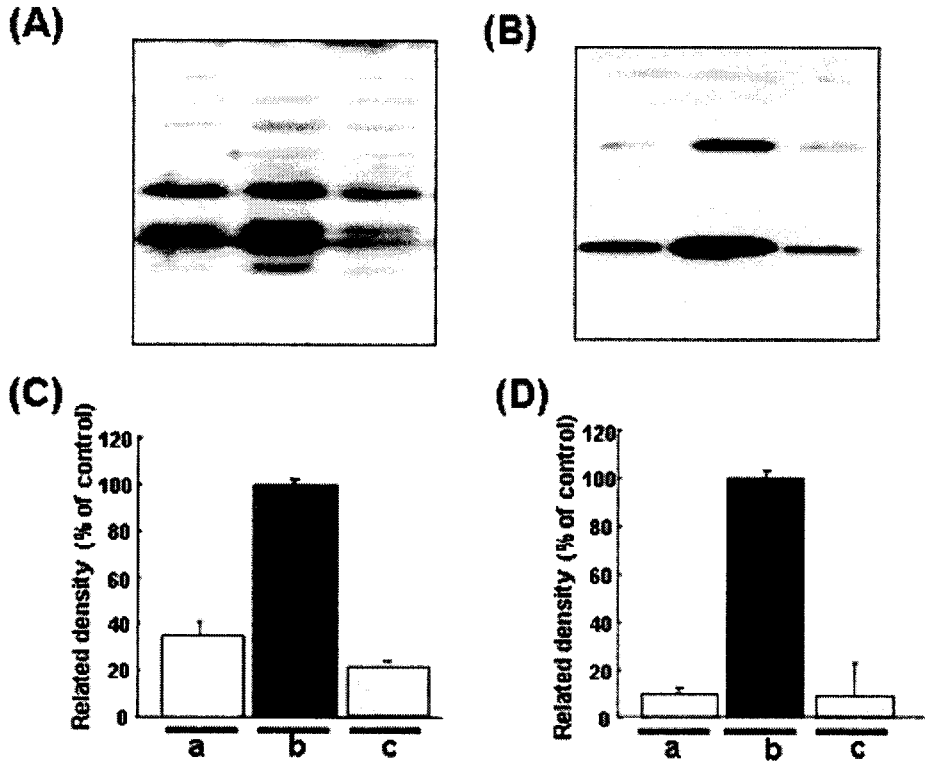

Fig. 4. Oral administration of "L-Skin Care" inhibits chronic UVB irradiation-induced expressions of MMP-3 (A, C) and MMP-13 (B, D) in hairless mice skin.
a, non-UVB exposed; b, Vehicle + UVB; c, "L-Skin Care"+UVB.

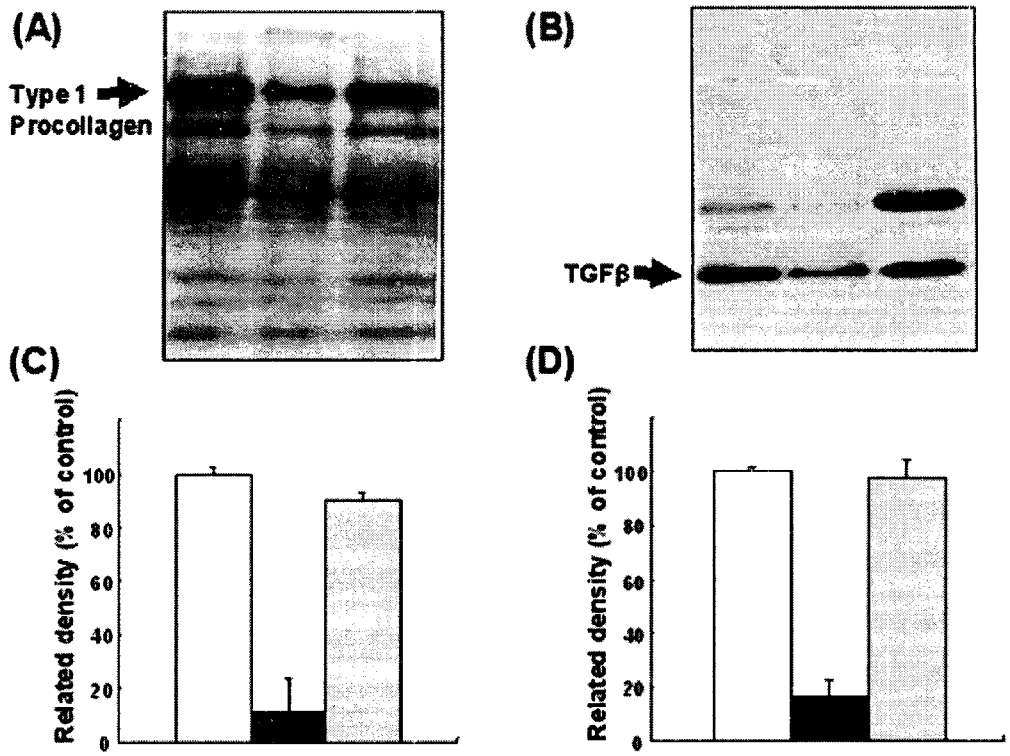

Fig. 5. Oral administration of "L-Skin Care" prevents UVB irradiation induced expressions of procollagen (A, C) and transforming growth factor-β2 (B, D) in hairless mice skin. a, non-UVB exposed; b, Vehicle + UVB; c, "L-Skin Care"+UVB.