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Anti-aging and Anti-inflammation Effects of Natural Mineral Extract on Skin Keratinocytes

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Abstract In this study, we investigated the effects of a natural mineral extract on damaged skin keratinocytes. We have focused on the possible effects of the natural mineral extract on UV-irradiated normal human skin keratinocytes. Human keratinocytes were UV-irradiated and were treated with 0.1, 1.0, or 10% of natural mineral extract. Study controls included non-UV-irradiated cells, UV-irradiated cells (no extract), and UV-irradiated cells treated with 25 nM ascorbic acid. Cell viability following UV irradiation was significantly higher in the groups treated with the natural mineral extract at doses of 0.1, 1.0, and 10% than in the control group with UV irradiation only. The UV-irradiated cells demonstrated increased secretion of laminin relative to non-irradiated controls. We found that treating these UV-irradiated keratinocytes with natural mineral extract reduced vacuole size and number, and it dose-dependently increased laminin secretion and reduced IL-2 synthesis. Also, MMP-1 and MMP-2 activity were significantly less in cells treated with natural mineral extract than in UV-irradiated controls. The UV irradiation reduced MMP-1 levels to 45±5 ng/mL from 130± 5 ng/mL. In addition, MMP-2 production in keratinocytes was significantly reduced by 11±1 ng/mL compared to the UV-irradiation control. In conclusion, the results of this work suggest that natural mineral extract has effects on keratinocytes damaged by UV exposure.

Keywords: actinomycin mineral, keratinocyte, UV irradiation, cytokine

INTRODUCTION

Environmental or exogenous factors such as ultraviolet (UV) radiation, wind, and smoke contribute to the extrinsic aging of skin. UV is a major environmental factor that alters the homeostasis of the skin by affecting the survival, proliferation, and differentiation of cell types. The effects of UV on the skin include direct damage to DNA, apoptosis, and growth arrest [1]. This type of aging, combined with intrinsic, or chronological aging, results in degeneration of the skin barrier, development of rhytides, discoloration, possible malignant degeneration, and other changes [2,3].

Photoaging is related to severe UV-induced damage of the dermal extracellular matrix. The patho physiological process of photoaging and skin aging derives largely from aberrant regulation of a multitude of finely-tuned molecular mechanisms, which have evolved to maintain the structural integrity of the skin's connective tissue [4]. Dermal photo damage is characterized by wrinkle formation, loss of recoil capacity, increased fragility, and impaired wound healing [5].

Recently, consumer and media attention has focused specifically on products utilizing "natural" ingredients such as vitamins, minerals, and botanical extracts [6]. Many studies strongly suggest that some over the counter cosmetic ingredients (*i.e.* vitamins, minerals, and botanicals) have the potential to improve aging skin. L-ascorbic acid, the biologically active form has well-established roles in the human body as an antioxidant and as a cofactor for collagen synthesis [7,8]. Ascorbate participates in the hydroxylation of procollagen, and studies show that it may also stimulate collagen synthesis directly by activating collagen transcription and stabilizing procollagen mRNA [7,9]. Other popular "natural" cosmetic ingredients applied to aging skin, but for which no references were found in a Medline search, are

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Table 1. Other popular natural ingredients for aging skin without published studies in a Medline search

Ingredient	Proposed mechanism
Seaweed extract	Antioxidant activity
Peppermint extract	Promotes circulation and relieves swelling
Algae extract	Unclear, possible antioxidant activity
Cucumber extract	Relieves swelling, suggested anti-inflammatory effects
Aloe vera	Anti-inflammatory effects
Wheat protein	Unclear
Witch hazel	Anti-inflammatory effects
Penthanol	Humectant

listed in Table 1.

Recent studies have reported an interaction between fibroblasts and keratinocytes following UV-irradiation. The results indicated MMP-1 production was induced, when conditioned medium from solar-stimulated radiation-exposed keratinocytes was added to human fibroblasts. Therefore, the aim of our current study was to clarify the roles of keratinocytes in ECM and MMP production after UV exposure.

Also, studies have shown the effects of Dead Sea minerals on keratinocyte cultures and human skin. Researchers found these minerals stimulate proliferation and mitochondrial activity, decrease the expression of some aging markers, and limit apoptotic damage after UVB irradiation [10]. Cellular extracts were analyzed by SDS-PAGE, which revealed increases in p16 and decreases in apoptosis inhibitor Bcl2. Deep-sea water (residing far below the ocean surface) was purified and enriched with vital minerals, including key elements (*e.g.* sodium, potassium, magnesium, calcium, chloride, and sulfate). These elements are necessary for vital functions of human cells [11]. Sea water has excellent effects on human skin. Comano (Trentino, Italy) spa's water (CW), thermal hypotonic water containing various electrolytes, and balneotherapy may beneficially affect the clinical manifestations of psoriasis *via* attenuation of the local deregulation of several cytokines (including IL-6 and VEGF-A isoforms) and of a concurrent, abnormal cell differentiation program entailing the expression of CK-16, among other proteins [12]. The beneficial effect of salt water baths and sun exposure on chronic skin diseases has been known for centuries. Pharmacological effects after immersion in salt solution were suggested in view of various *in vitro* and *in vivo* studies, modulatory effects on epidermal langerhans cells (magnesium, calcium), inhibition of 5-lipoosygenase enzyme (magnesium), inhibition of Th1 lymphocyte and keratinocyte-derived inflammatory cytokines (sulphate, selenium, and strontium), and antiproliferative effects on fibroblasts (bromide, magnesium) [13-16].

Loess, a yellow or yellowish-brown colored solid, is used as a building material, but was recently shown to have positive effects on the human body. These results prompted new applications of the material. Loess contains various mineral compounds with antibiotic activities, and recent studies reported its effects on microorganisms and plants [17-19].

On average the contents of loess are Si, Al, Fe, Mg, Ca, Na, K, and Mn. Researchers examined the impact of yellow soil on growth and physicochemical characteristics of soybean sprouts. The weight and length of soybean sprouts increased rapidly and nucleotides (such as UMP, CMP, and AMP), Hx, and soluble free sugars like sucrose, raffinose, stachylose were detected in the sprouts [17].

In this study we investigated the effect of natural mineral extract on cell growth and cell morphology, and on the expression of laminin, MMP-1, MPP-2, and IL-2 in UV-irradiated normal human keratinocytes.

MATERIALS AND METHODS

Sample Preparation

The natural mineral extract was prepared using the following methods. Loess (970 g) (Gochang Hwangto, Korea) and 30 g of sodium hydroxide were mixed, and placed in a melting pot. The mixture of loess and sodium hydroxide was melted for 6 h at 1400ºC, using an electric furnace. The melted liquid was cooled at room temperature and crushed to a particle size $\leq$ 10 μm in a ball mill and Micro Jet Mill (Jei Bunchai, Korea).

The administering solution was made as follows: nano-sized (diameter) particles of the sample were dissolved in distilled water at a concentration of 1% (v/v). After 24 h, the natural mineral extract supernatant was separated.

Chemical Analysis

The chemical compositions of SiO_2-ionized loess and untreated loess were analyzed using ICP OES (PerkinElmer DV 3300, USA), according to KS L 4007: 2006 of Korean Standards Association. Pb, As, Cd, and Hg were analyzed by ICP-OES after acid digestion, and Cr^{+6} was analyzed by UV/VIS Spectrophotometer (Shimadzu, Japan) at 540 nm absorbance after digestion for 1 h at 90ºC using an alkaline solution containing Na_2CO_3, NaOH, $MgCl_2$, and phosphate buffer, according to the US EPA (Environment Protection Agency) 3060A method.

Primary Culture of Skin Keratinocytes

Normal human skin was aseptically isolated from a circumcised neonatal foreskin at Chung-Ang University Hospital (Yong San, Korea). The donated foreskin was immersed in Dulbecco's modified Eagle medium (DMEM) containing antibiotic and antimycotic agents (100 U/mL penicillin G sodium, 100 U/mL streptomycin sulfate, and 0.25 μg/mL amphotericine B; WellGen, Korea) at 4ºC. The piece of foreskin was immersed in DMEM containing 1.4 U/mL Protease (Dispase, Sigma Chemical Company, St. Louis, MO, USA) for 16 h at 4ºC. The epidermal layers were mechanically stripped and keratinocytes were isolated from the epidermis by 0.05% trypsin treatment for 15 min at 37ºC. Following

this enzymatic treatment, DMEM supplemented with 10% fetal bovine serum (FBS) was added to the cell suspension. This diluted suspension was centrifuged for 5 min at 800 rpm. The cultivated keratinocytes were inoculated into the culture medium. The cells were grown in keratinocyte serum-free medium (K-SFM, Gibco BRL) consisting of MCDB 153 medium supplemented with insulin (0.005 g/L), hydrocortisone (0.074 mg/L), triiodothyronine (0.0067 mg/L), bovine pituitary extract (50 mg/L), and epidermal growth factor (EGF, 5 μg/L) at 37ºC in a 5% CO_2 incubator.

UVB Irradiation

Normal human keratinocytes were grown in monolayer cultures using K-SEM supplemented with bovine pituitary extract (25 mg, GIBCO) and recombinant epidermal growth factor (2.5 μg, GIBCO). For these experiments the human keratinocytes were seeded at a density of 1×10^5 cells/well in 6-well plates and irradiated after 1 day. Prior to irradiation, cells were washed with PBS and irradiation was performed through a thin film of PBS *via* exposure to 20 mJ/cm^2 of UVB at 312 nm dose, as measured with an SX-312 research radiometer (UVitec. Ltd.,). We examined the cells after UV irradiation and keratinocyte treatments were repeated 3 times in a week.

Human keratinocytes were treated with natural mineral extract at 0.1, 0.5, or 1% or with 25 nM ascorbic acid. Various assays were performed after 48 h of UV irradiation.

Cell Viability Assay

Cell viability was measured using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma, USA) assay. For the MTT assay, serum-free DMEM was added to each well and supplemented with MTT (0.33 mg/mL). The plates were incubated in the dark at 37ºC in an atmosphere containing 5% CO_2 for 2 h and the supernatant was aspirated. Isopropyl alcohol (1 mL) containing 0.04 N HCl was added and the dish was shaken slowly for 15 min. The absorption was measured at 540 nm.

Morphological Examination and Immunohistochemistry

The cultured keratinocytes were fixed for 1 h at 4ºC using 10% neutral buffered formalin and immunohistochemical staining was performed in the culture dishes. The endogenous peroxidase was blocked using 0.03% H_2O_2, followed by the nonspecific reaction of bovine serum albumin and non-immune serum with the respective monoclonal antibody of laminin (NeoMarker, Lab Vision Corp, Fremont, CA). Finally, it was followed by the standard immunohistochemical procedure with anti-goat or anti-rabbit immunoglobulin, using the ABC (Avidin-Biotin peroxidase complex) method.

Western Blot Analysis

Cells were lysed in cell lysis buffer (62.5 mM, Tris-HCl (pH 6.8); 2%, SDS; 5%, β-mercaptoethanol; 2 mM, phenylmethyl-sulfonyl fluoride and protease inhibitors (Complete^{TM}, Roche, Mannheim, Germany), 1 mM, Na_3VO_4; 50 mM, NaF; and 10 mM, EDTA). Ten micrograms of protein was separated using SDS-polyacrylamide gel electrophoresis and blotted onto PVDF membranes, which were blocked with 5% dried milk in Tris-buffered saline containing 0.5% Tween 20. A polyclonal anti-laminin antibody (kindly provided by Prof. Chung, Seoul National University) was used as the primary antibody. Blots were incubated with the primary antibodies at a dilution of 1:1,000 and they were further incubated with horseradish peroxidase-conjugated secondary antibody.

MMP-1 and MMP-2 assays

Proteins are crucial factors for enhancing target protein expression in the induction medium [20]. To determine the amount of MMP-1 and MMP-2 secreted into culture media by human keratinocytes, we used human MMP-1 kits (R&D, DMP100) and MMP-2 kits (R&D, DMP200) were used for an ELISA immunoassay. The assay procedure was performed manually and the absorption was measured at 450 nm.

IL-2 assay

After UV irradiation and treatment, keratinocytes were incubated with K-SFM without serum. Supernatants were harvested for 48 h after the treatment. IL-2 is constitutively expressed in keratinocytes, when cultured in keratinocyte growth medium. IL-2 activity was measured by interleukin-2 ELISA (BioSource, C0021C). The assay procedure was performed manually and the absorption was measured at 450 nm.

Statistical Analysis

The data was statistically evaluated using the Student's *t*-test and data are given as means ± SD. The difference between the means was considered significant when $p \leq 0.05$.

RESULTS

Mineral Composition of Natural Mineral Extract

The contents of the mineral elements in untreated loess and SiO_2 ionized loess are listed in Table 2. The SiO_2 ionized loess was composed of amorphous silica (SiO_2), aluminum oxide (Al_2O_3), ferric oxide (Fe_2O_3) 2.61 %, titanium oxide (TiO_3) 0.4%, and calcium oxide (CaO) 0.42%, as shown in Table 2. Other common natural mineral components include magnesium oxide (MgO), potassium oxide (K_2O), natrium (Na_2O), and phosphorus oxide (P_2O_5).

Cell Viability and Morphology

The effect of UV irradiation on kerationcyte cell viability was assayed after 48 h of UV irradiation. As shown in Fig. 1, there was a significant difference between the control and

Table 2. Composition of untreated loess and SiO_2-ionized loess (%)

Component	Result (%)	
	Untreated loess	SiO_2-ionized loess
SiO_2	81.04	84.41
Al_2O_3	1.43	1.50
Fe_2O_3	5.82	2.61
CaO	< 0.001	0.42
MgO	< 0.001	2.16
K_2O	2.38	2.87
Na_2O	0.20	4.95
TiO_2	0.76	0.38
Ig. loss[a]	8.28	0.64
Pb	n.d.[b]	n.d.
As	n.d.	n.d.
Cd	n.d.	n.d.
Cr^{+6}	n.d.	n.d.
Hg	n.d.	n.d.

[a] Ignite loss. [b] not detectable.

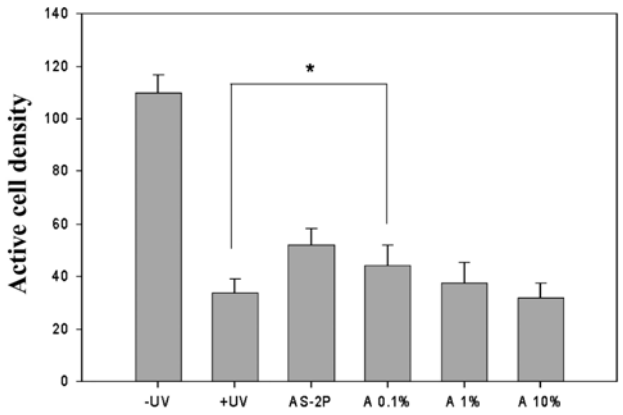

Fig. 1. Effect of natural mineral extracts on skin keratinocyte growth. A 0.1%: 0.1 % (v/v) natural mineral extract treatment after UV-irradiation, A 1%: 1% natural mineral extract treatment after UV-irradiation, and A 10%: 10% natural mineral extract treatment after UV-irradiation. *$p \leq 0.05$.

the treatment groups. Natural mineral extract treatment (0.1%) appeared to significantly increase cell growth as compared with UV-irradiated controls.

This experiment indicated that treatment with natural mineral extract did not cause toxicity and tended to promote recovery of UV-irradiated keratinocytes at low doses of natural mineral extract. Cell viability following UV irradiation was significantly higher in the groups treated with natural mineral extract at doses of 0.1 and 1.0% in comparison to the control group with UV irradiation only. However, we observed no statistically significant increase of cell activity in the high dose treatment.

We observed that large cytoplasm vacuoles formed following the UV irradiation of human skin keratinocytes. Fig. 2B shows that these vacuoles were reduced in the AS-2P treatment group, when compared with the group that received UV irradiation only (arrow: vacuoles). The results also demonstrated that vacuole size and number were reduced in a dose-dependent manner, as natural mineral extract levels were increased.

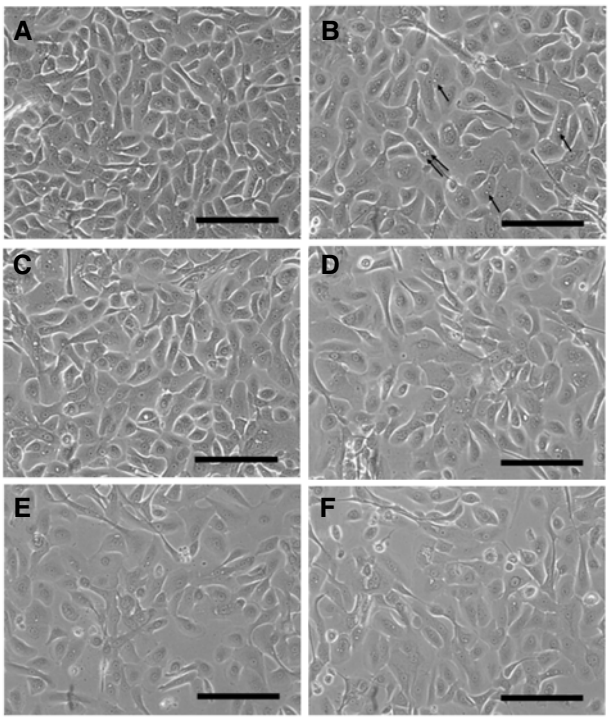

Fig. 2. Phase-contrast photography of human skin keratinocytes. (A) No UV irradiation, (B) UV-irradiation, (C) As-2p treatment after UV-irradiation, (D) 0.1% natural mineral extract treatment after UV-irradiation, (E) 1% natural mineral extract treatment after UV-irradiation, and (F) 10% natural mineral extract treatment after UV-irradiation. (Arrow: vacuoles, Original magnification: x 100, Scale bar: 200 μm).

Laminin Levels Increase in Natural Mineral Extract Treated Keratinocytes

Laminin staining showed that natural mineral extract treatment affects the synthesis of laminin within the cell. In response to UV exposure, a reduction in laminin protein could be weakly detected by cell staining (Fig. 3A).

However, cultures treated with AS-2P demonstrated moderate amounts of laminin staining (Fig. 3C). We observed no significant difference in the detected amount of laminin between the low and high dose natural mineral extract treatments.

Immunohistochemistry results show that natural mineral extract increased laminin staining in UV irradiated keratinocytes. Western bolt analysis also showed a natural mineral extract dose-dependent increase in laminin production in the

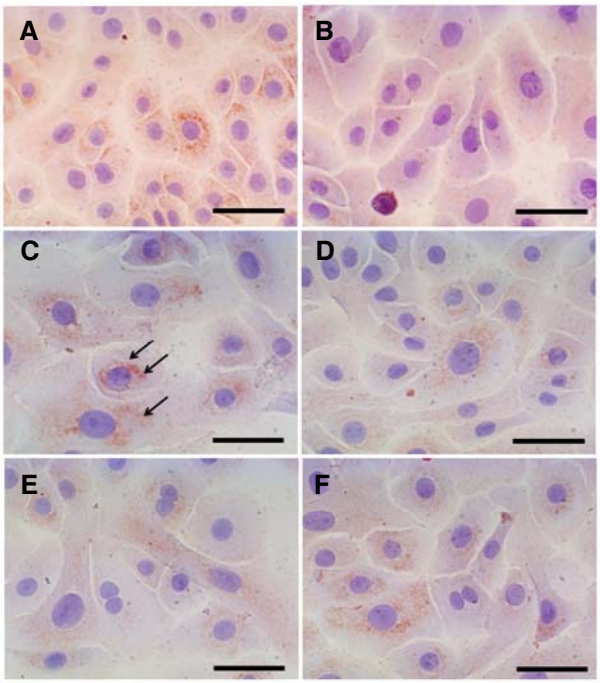

Fig. 3. Immunohistochemical staining of laminin in skin keratinocytes. (A) No UV irradiation, (B) UV-irradiation, (C) As-2p treatment after UV-irradiation, (D) 0.1% natural mineral extract treatment after UV-irradiation, (E) 1% natural mineral extract treatment after UV-irradiation, (F) 10% natural mineral extract treatment after UV-irradiation. (Brown color: secreted laminin Original magnification: × 400, Scale bar: 100 μm.)

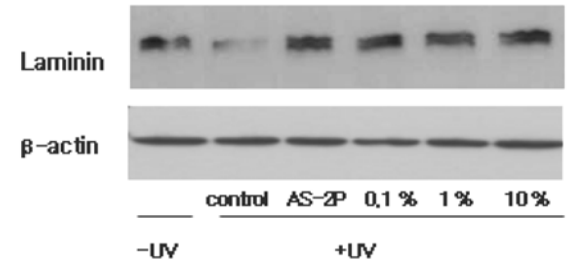

Fig. 4. Effect of natural mineral extracts on the laminin secretion of keratinocytes after UV irradiation (Western blot). A 0.1%: 0.1 % (v/v) natural mineral extract treatment after UV-irradiation, A 1%: 1% natural mineral extract treatment after UV-irradiation, and A 10%: 10% natural mineral extract treatment after UV-irradiation.

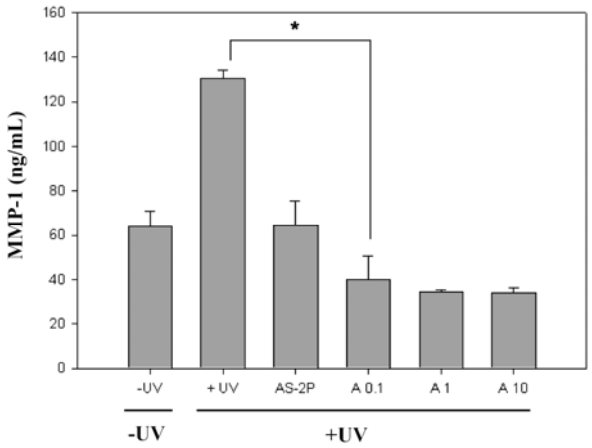

Fig. 5. Effect of natural mineral extracts on the MMP-1 secretion of keratinocytes after UV irradiation. A 0.1%: 0.1 % (v/v) natural mineral extract treatment after UV-irradiation, A 1%: 1% natural mineral extract treatment after UV-irradiation, and A 10%: 10% natural mineral extract treatment after UV-irradiation. *$p \leq 0.05$.

treated cells. These results indicate that natural mineral extract treatment increased laminin expression in human skin keratinocytes (Fig. 4).

Expression of MMPs in Irradiated Human Skin Keratinocytes

Keratinocytes, the major target of UV radiation, play a central role in the inflammatory and immunomodulatory changes observed after UV exposure; these changes are at least partly due to the UV-induced release of cytokines and arachidonic acid metabolites [21]. Therefore, we investigated the mechanism by which natural mineral extract inhibits its MMP-1 and MMP-2 expression.

As shown in Fig. 5, concentration dependence of the effect of natural mineral extract treatment on cell MMP-1 activity was significantly less than in UV irradiated control levels. The amount of MMP-1 measured after UV irradiation was 130 ± 5 ng/mL, which was higher than for non-irradiated cells (70 ± 10 ng/mL). As-2p significantly protected cells against the secretion of MMP-1 after UV exposure, and natural mineral extract treatment reduced this production by 45 ± 5 ng/mL at 0.1%, 40 ng/mL at 1% and 10%.

MMP-1 secretion by UV-irradiated human keratinocytes is reduced by natural mineral extract treatment in a dose dependent manner.

We next investigated the effects of natural mineral extract treatment on MMP-2 expression in UV-irradiated human keratinocytes. Fig. 6 shows that UV exposure resulted in the highest levels of MMP-2 secretion (15 ± 1 ng/mL) as detected by ELISA. The AS-2p treatment group significantly reduced MMP-2 production in keratinocytes by 11 ± 1 ng/mL compared to the UV irradiation control ($p \leq 0.05$). Furthermore, treatment with natural mineral extract induced a significant decrease in MMP-2 expression compared to the untreated control. Together these observations indicate that natural mineral extract treatment reduces UV-induced MMP-1 and MMP-2 secretion by keratinocytes.

Natural Mineral Extract Decreases the Synthesis of IL-2

We also investigated the effects of natural mineral extract

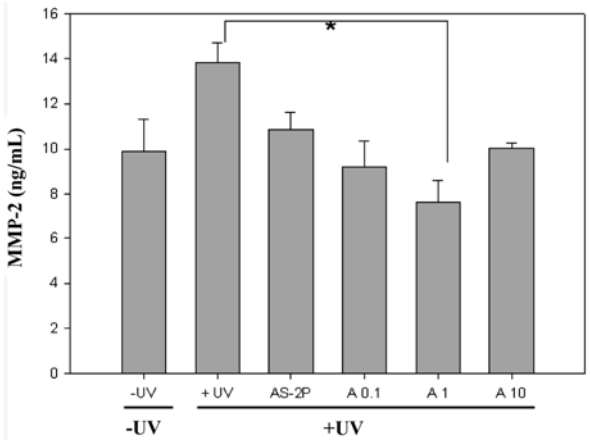

Fig. 6. Effect of natural mineral extracts on the MMP-2 secretion of keratinocytes after UV irradiation. A 0.1%: 0.1 % (v/v) natural mineral extract treatment after UV-irradiation, A 1%: 1% natural mineral extract treatment after UV-irradiation, and A 10%: 10% natural mineral extract treatment after UV-irradiation. *$p \leq 0.05$.

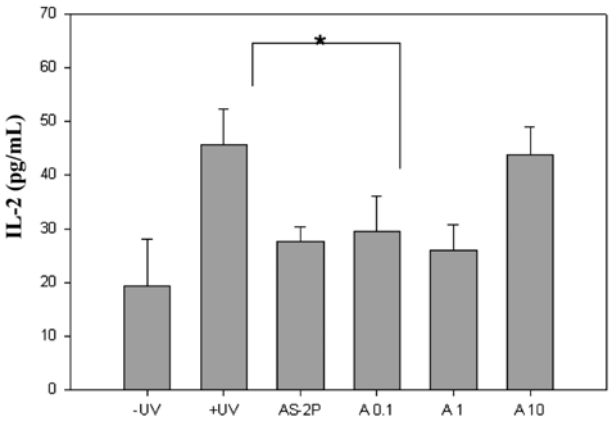

Fig. 7. Effect of natural mineral extracts on the IL-2 secretion of keratinocytes after UV irradiation. A 0.1%: 0.1% (v/v) natural mineral extract treatment after UV-irradiation, A 1%: 1% natural mineral extract treatment after UV-irradiation, and A 10%: 10% natural mineral extract treatment after UV-irradiation. *$p \leq 0.05$.

treatment on IL-2 secretion in normal skin keratinocytes. Natural mineral extract significantly protected keratinocytes against inflammation following exposure. As shown in Fig. 7, IL-2 secretion in normal skin keratinocytes is reduced by natural mineral extract in a dose-dependent manner. AS-2P reduced IL-2 production approximately 30 pg/mL, compared to the reduction following UV irradiation (45 pg/mL). Natural mineral extract concentrations of 0.1 and 1% inhibited IL-2 production by keratinocytes after UV exposure.

DISCUSSION

Loess has long been identified with the life and culture of Asian. Colloquially, it is said that moderate loess consumption is beneficial to health and skin. However, a detailed analysis for the efficacy of loess treatment on skin has not been conducted. Therefore, in this study, we have focused on finding additional effects of loess on skin.

Typical contents of loess are Si, Al, Fe, Mg, Ca, Na, K, and Mn. Our samples included amorphous silica (SiO_2), aluminum oxide (Al_2O_3), ferric oxide (Fe_2O_3), titanium oxide (TiO_3), calcium oxide (CaO), magnesium oxide (MgO), potassium oxide (K_2O), natrium (Na_2O), and phosphorus oxide (P_2O_5) (Table 2). In our study natural mineral extract supernatant was composed of ionized minerals.

Several research reports have described that bathing in Dead Sea benefitted skin by improving skin barrier function, increasing stratum corneum hydration, reducing skin roughness and inflammation [22], and dramatically reducing the inflammation in psoriasis [23]. All of these processes are, to various extents, relevant to the condition of aged skin. Climatotherapy at the Dead Sea has been used successfully for more than 25 years for treating moderate to severe psoriasis [24-27]. Treatment includes daily bathing in Dead Sea water and sunlight exposure. Histologically there was an overall reduction in malpighian layer thickness by 63.4% and T-lymphocytes were almost totally eliminated from the epidermis [28]. In addition, the treatment of combined 5% NaCl solution and UVB radiation was suggested as a valuable treatment alternative for severe atopic, particularly in younger populations [29].

In addition, recent studies investigated various minerals that have been used in the treatment of skin disease. Consequently, we designed this study to investigate the efficacy of natural mineral extract for treating damaged human skin keratinocytes.

UV primarily acts on the epidermal basal cell layer of the skin, inducing direct and indirect adverse biological effects. In particular, the formation of photoproducts, cell cycle growth arrest, photoaging, photocarcinogenesis, and immunodepression can be attributed to UV irradiation [30-32]. UVB penetrates the epidermis and to a lesser extent the upper part of the dermis. Fig. 2 shows the morphologic change caused by UV irradiation in keratinocytes. However, topical treatment with AS-2P and natural mineral extract significantly reduced the morphologic change. A natural mineral extract did not show a significant dose-dependent change in morphology. In addition, as shown in Fig. 1, natural mineral extract activated cell growth of UV irradiated cells at lower concentrations (0.1% v/v) and higher concentrations (10% v/v). Therefore, Figs. 1 and 2 demonstrate that natural mineral extract protects cells and reduces toxicity.

Keratinocytes are the major target of UV, and they play a central role in the inflammatory and immune modulatory changes accompanying UV exposure, which are at least partly driven by the UV-induced release of cytokines [21]. Cytokines and chemokines undoubtedly play pivotal roles in immunologic regulation in the human body, and are involved in the induction of proliferation, differentiation, and

cell death. For example, interleukin (IL)-1β, IL-6, IL-8, and IL-12 are involved in inflammatory responses *in vivo*. IL-1β is mainly produced by monocytes, dendritic cells, B-lymphocytes, and normal keratinocytes. Keratinocytes can produce IL-1β, when stimulated, but these cells constitutively express IL-1α [33]. This indicates that dermal damage induced by UV irradiation might not only be due to specific, direct effects on the dermis, but may also result from indirect activation of epidermal cytokines [34]. Following UV irradiation, MMPs are increasingly produced by fibroblasts and epidermal cytokines are produced by keratinocytes. UV irradiation activates MAP kinase signaling transduction pathways (including EPK, JNK, and p38) through stimulation of growth factors and cytokine receptors on the surface of keratinocytes. For instance, IL-2, a cytokine that stimulates growth of immune T cells, normally is depleted in the extracellular environment by this mechanism, leading to cessation of signaling.

In this study, cell culture media was harvested for protein level analysis; low levels of IL-2 were detected in keratinocytes during treatment. However, natural mineral extract did not effectively reduce IL-2 expression at high concentrations (10% v/v). These results suggest that natural mineral extract is more active at low concentrations than high concentrations.

UV irradiation activates growth factor and cytokine receptors on the surface of keratinocytes. Activated receptors stimulate signal transduction cascades that activate transcription factor AP-1, which in turn stimulates transcription of matrix metalloproteinase (MMP) genes [35]. MMPs are responsible for remodeling and degrading collagenous extracellular matrices in connective tissues [35]. The MMPs, of which there are more than 24 in humans, are a family of zinc binding endopeptidases that play a fundamental role in normal processes such as the remodeling of extracellular matrix structures during wound healing [36], dermal photoaging [37], and several pathologies (*i.e.* carcinogenesis) [38].

We found that the effect of natural mineral extract on MMP-1 (metalloproteinase-1) production in UVB-irradiated keratinocytes was twice that in non-irradiated keratinocytes. However, natural mineral extract treatment group suppressed the MMP-1 secretion compared with AS-2P treatment group in keratinocyte. Fig. 6 shows that natural mineral extract suppressed MMP-2 expression in UV-irradiated keratinocytes.

Moreover, UV-induced damage can lead to degeneration of the skin's basement membrane (including an accumulation of degenerative elastic material) and can induce apoptosis. Basement membrane changes in human skin exposed to UV irradiation were previously studied by light and electron microscopy, and by using antibodies against human collagen IV and laminin. Laminin, the principal multi adhesive matrix protein in basal laminae and domains of the laminin subunit mediated Ca^{2+}-dependent binding to specific carbohydrates on certain cell surface molecules.

Our experiments showed that natural mineral extract treatment induces high levels of laminin expression (Fig. 4.). After UV irradiation, absolute laminin level were higher in natural mineral extract treated keratinocytes and the observed effects appear to be concentration-specific.

CONCLUSION

In this work we have investigated the *in vitro* capability of natural mineral extract to protect human skin cells against UV irradiation. Keratinocytes are the major target of UV radiation, and play a central role in the inflammatory and immunomodulatory changes observed after UV exposure. Our study demonstrated a change in laminin, and MMP expression and IL-2 secretion after UVB irradiation in keratinocytes. We further demonstrated that the dose-dependent effects of natural mineral extract treatments increased cell growth and laminin expression. In addition, MMP-1, MMP-2, and IL-2 secretion decreased in UV-irradiated keratinocytes. Therefore, our results suggest that this natural mineral extract has significant potential for use in medical or cosmetic treatments following UV exposure.

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