ANTI-WRINKLE EFFECT OF LEG BONE HYDROLYSATES AND OIL FROM JEJU CROSSBRED HORSES

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Abstract – This study investigated the anti-wrinkle activity of horse leg bone hydrolysates less than 3 kDa (HL) and horse oil (HO) on ultraviolet-induced photoaging in hairless mice (Skh-1). Skin wrinkles were induced by UVB-irradiation for 10 weeks on the back of Skh-1 hairless mice three times a week. Total 70 mice were divided into 7 groups: –NC (normal group), +UC (UV control), +HLL (dietary HL, 500 mg/kg/d BW), +HLH (dietary HL, 1000 mg/kg/d BW), +HO (spreading HO), +HOHLL (spreading HO and dietary HL, 500 mg/kg/d BW), +HOHLH (spreading HO and dietary HL, 1000 mg/kg/d BW). Wrinkle formation, skin moisture, transepidermal water loss (TEWL), wrinkle depth and epidermal thickness were analyzed. +UC induced epidermal barrier dysfunction including a decrease in skin moisture, an increase in TEWL, skin wrinkle depth and epidermal thickness. The skin moisture of +HOHLH (63.32%) was higher than in +UC (52.26%). TEWL was decreased in HL and HO. In +HLH (17.52 μm), the wrinkle depth significantly lower than in +UC (24.74 μm). The epidermis thickness in +UC was higher than that in +HLL, +HLH, +HO, +HOHLL and +HOHLH. These results indicate that HL and HO exerts anti-photoaging activities by improving wrinkle formation and dryness.

Key Words – Hairless mice, UVB, Skin

I. INTRODUCTION

Studies of anti-skin aging have focused on development of functional materials. Skin is essential for human survival. Damage to human skin due to repeated exposure to ultraviolet (UV) radiation (photoaging) and damage occurring as a result of the passage of time (intrinsic aging) are considered to be distinct entities rather than similar skin aging processes [8]. UVB is thought to be a major contributor to photoaging [5]. UVB damages skin tissues and cells both indirectly and directly through inflammation and production of reactive oxygen species [1]. Currently, commercial products use collagen extracts from swine flesh and fish, and have low polymer molecular weights with molecule sizes of 30 kDa or 1-5 kDa [9]. In our previous study, antioxidant effect was observed in the low molecular weight peptide fraction of less than 3 kDa isolated from horse leg bone extracts via enzyme hydrolysis [3]. Also our previous study indicated that low molecular weight peptides from Jeju horse leg bone hydrolysates has potential utility as an anti-oxidant and anti-wrinkle agent in the food and cosmetic industry [2]. Aslo, horse oil cosmetics such as cream, body cream and lotion are come to the market in Japan and Korea. Horse oil has an advantage that is readily absorbed into human skin because it has very similar composition to human epidermal lipids [4].

However, there are lack of information about the effects of HL and HO on anti-wrinkle effect in vivo. Therefore, in the present study, we administered HL and/or spread purified HO to hairless mice after exposure of UVB irradiation, and examined their anti-wrinkle effects.

II. MATERIALS AND METHODS

Materials Horse leg bone hydrolysates was prepared according to the method of Kim et al [3]. The horse oil refined through degumming, deacidification and deodorization.

Fatty acid composition Fatty acid composition of horse oil was analyzed by gas chromatography (Agilent 6890N, Agilent Technologies, USA). Aliquots of the hexane extract were injected in splitles mode onto a HP-Innowax column (25um film thickness, 30m * 0.32mm, Agilent Technologies, USA). The injector temperature was 230 °C, detector at 250 °C, oven at 180 °C, then 180–230 °C at 1.5 °C per min, 230 °C for 5 min. The carrier gas was N_2 and the flow rate was 1 ml/min.

Animals and experimental group Forty nine female hairless mice of Skh-1 were randomly divided into the seven groups as shown in Table 1. Feed intake and body weight were measured thrice and once a week, respectively. Animals in the normal control and UV control were given normal saline at the same volume. All mice, except the normal control, were irradiated with the same UV source. After supplementation for 10 weeks, forty nine mice were sacrificed by cervical dislocation and tissue samples were collected. The intensity of UVB irradiation (Philips, Germany) was regulated using UV-Radiometer (Vilber Lourmat, France). The initial dose was set at 36 mJ/cm^2 at, which was subsequently increased to 54, 72, 108, 144, 162, and 180 mJ/cm^2 at 1-week intervals and finally to 216 mJ/cm^2 at weeks 8, 9 and 10.

Table 1. Experimental design

Treatment		UVB	Feed	Spreading amount
Normal control	-NC	-	Basal diet	-
UV control	+UC	+	Basal diet	-
Horse leg bone hydrolysates less than 3kDa (Low)	+HLL	+	500mg/kg B.W	-
Horse leg bone hydrolysates less than 3kDa (High)	+HLH	+	1000mg/kg B.W	-
Horse oil	+HO	+	Basal diet	100 $\mu\ell$
Horse oil + Horse leg bone hydrolysates less than 3kDa (Low)	+HOHLL	+	500mg/kg B.W	100 $\mu\ell$
Horse oil + Horse leg bone hydrolysates less than 3kDa (High)	+HOHLH	+	1000mg/kg B.W	100 $\mu\ell$

Measurement of TEWL and skin hydration TEWL and hydration were measured on the dorsal skin. TEWL was measured quantitatively using a Tewameter (TM300, Courage+Khazaka, Cologne, Germany). Skin hydration was measured using a corneometer CM825 (Courage+Khazaka, Cologne, Germany).

Wrinkle measurement Wrinkle improvement was evaluated by measuring total wrinkle depth using the Visioline VL650 (Courage + Khazaka GmbH, Cologne, Germany).

Histological examination The dorsal skins remove from Skh-1 were subjected to hematoxylin and eosin. Through tissue evaluations, the epidermis thickness of the dermal layer was observed.

Statistical analysis Data were analyzed using the SAS software (ver. 9. SAS Institute Inc., USA). Mean separation was conducted using Tukey's multiple range test ($p < 0.05$). All tables indicate the mean values and the standard error of the means (SEM).

III. RESULTS AND DISCUSSION

Fatty acid composition of horse oil Palmitoleic acid in purified horse oil (9.52%) was higher than in crude horse fat (4.81%)(data not shown). Palmitoleic acid is part of skin lipids and provides a building block for wounds, skin scratches and healing burns [7].

Body weight, weight gain and feed efficiency ratio of hairless mice There are no significant differences on all experimental group for initial (23.31-23.69 g) and final (27.57-29.49 g) body weight. The weight gain and feed efficiency ratio (FER) were ranged 0.06~0.09 mg/day and 0.015~0.021, respectively. Also there was no significant difference on FER and weight gain (data not shown) in all experimental group.

Organ weight of hairless mice Liver (4.33-4.75%), spleen (4.40-4.47%), kidneys (1.36-1.44%) and lung (0.56-0.63%) showed no significant difference in all experimental groups (data not shown).

Mice skin moisture Changes in the skin moisture capacity of hairless mice after UV irradiation were measured (Table 2). The value of skin moisture in –NC (62.83%) was significantly higher than in the +UC(52.26%) at week 10. In group +HLL (57.89%), the skin moisture was higher than in group +UC. The skin moisture of group +HLH (60.43%), +HO (61.61%), +HOHLL (62.50%), +HOHLH (63.32%) was similar to that of the -NC. The studies confirmed that HL and particularly

HO could increase moisture content that the decline due to UV rays.

Table 2 Effect of horse leg bone hydrolysates and horse oil spreading on skin moisture of hairless mice (%)

Treatments[1)]	Week 0	Week 10
-NC[1)]	59.16[a]	62.83[ab]
+UC	58.08[a]	52.26[c]
+HLL	59.13[a]	57.89[b]
+HLH	58.64[a]	60.43[ab]
+HO	57.69[a]	61.61[ab]
+HOHLL	60.01[a]	62.50[ab]
+HOHLH	58.83[a]	63.32[a]
SEM	1.280	1.191

[a-c]Means within same column with different superscript letters differ significantly at $p<0.05$.
[1)]-NC, Normal control; +UC, UV control; +HLL, Horse leg bone hydrolysates less than 3kDa (Low); +HLH, Horse leg bone hydrolysates less than 3kDa (High); +HO, Horse oil; +HOHLL, Horse oil +Horse leg bone hydrolysates less than 3kDa (Low); +HOHLH, Horse oil + Horse leg bone hydrolysates less than 3kDa (High)

Mice skin transepidermal water loss (TEWL) TEWL is a critical marker of epidermal barrier function. TEWL of experimental groups was shown in Table 3. The value of TEWL in control + UC (23.43 g/hm^2) was larger than the -UC (10.94 g/hm^2) at week 10. In contrast, this increase in TEWL was significantly suppressed by ingestion of +HLL (18.47 g/hm^2), +HLH (17.05 g/hm^2), +HO (19.08 g/hm^2), +HOHLL (18.57 g/hm^2) and +HOHLH (17.63 g/hm^2) at week 10.

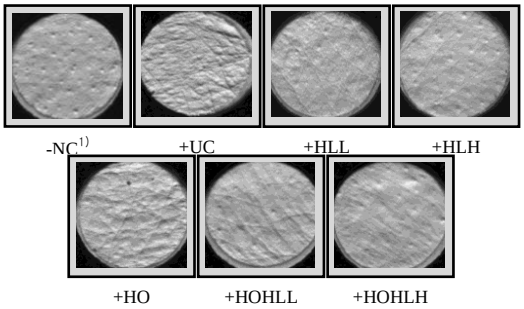
Table 3 The effect of horse leg bone hydrolysates supplementation and horse oil spreding on skin TEWL in UVB-irradiated hairless mice (g/hm^2)

Treatments[1)]	Week 0	Week 10
-NC	11.88[a]	10.94[c]
+UC	12.19[a]	23.43[a]
+HLL	11.33[a]	18.47[b]
+HLH	11.05[a]	17.05[b]
+HO	11.08[a]	19.08[b]
+HOHLL	10.88[a]	18.57[b]
+HOHLH	10.71[a]	17.63[b]
SEM	0.501	0.837

[a-c]Means within same column with different superscript letters differ significantly at $p<0.05$.
[1)]Refer to Table 2

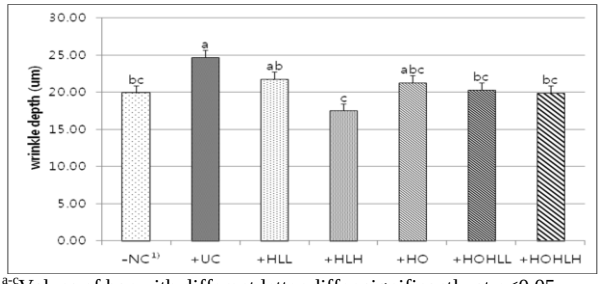
Wrinkle measurement and analysis of skin replicas Persisting UVB-irradiation was associated with UV-induces epidermal change such as roughening and wrinkle [6]. After UVB was repeatedly exposed to the skin of mice likely three times a week for ten weeks, the deep and coarse wrinkles were formed in +UC groups (Figure 1). However, spreading of HO reduced wrinkle formation. In particular, diets of HL effectively decreased wrinkle formation. Analysis of the replicas for the effects of the dorsal skin depth is shown in Figure 4. The dorsal skin wrinkle depth of the replicas in –NC (19.93 μm) was lower than in +UC (24.74 μm). UVB irradiation leads to wrinkle formation on the dorsal skin of +UV group. Also, the wrinkle depth in +UC was significant higher than that in +HLH (17.52 μm), +HOHLL (20.34 μm) and +HOHLH (19.94 μm). Similarly, Pyun et al. [6] indicated that UVB irradiation induced significant wrinkle formation (depth) in the dorsal skin of the UVB control group. Also, they reported that oral administration of collagen tripeptide reduced wrinkle formation (depth) induced by UVB irradiation.

Figure 1. Features of replicas taken from the dorsal skin of the UVB-irradiated hairless mice after supplementation leg bone extracts and spreading horse oil.



[1)]Refer to Table 2

Figure 2. Dorsal skin wrinkle depth (um) of UVB irradiated hairless mice after supplementation leg bone extracts and spreading horse oil.

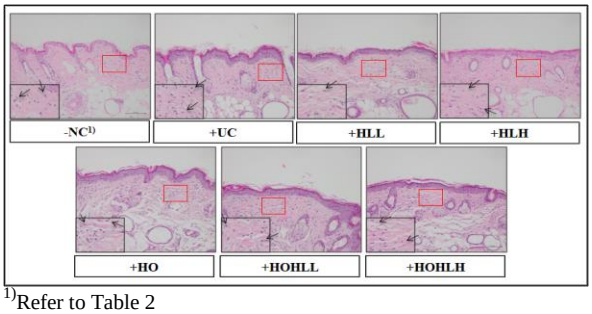


[a-c]Values of bar with different letter differ significantly at $p<0.05$.
[1)]Refer to Table 2

Histological examination and epidermal thickness Based on H&E- stained dorsal skin (Figure 3), the thickness of the epidermis (stained in dark purple) was observed (Figure 4). The epidermals of HL and HO treatment hairless mice were thinner than the negative control mice skin.

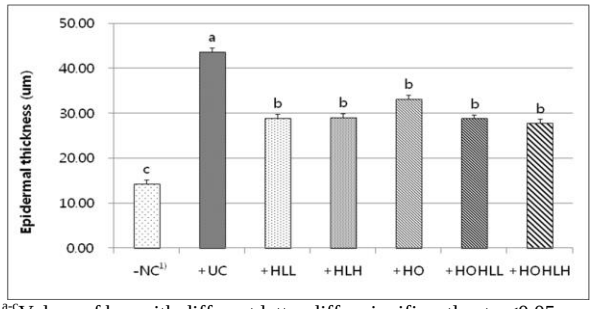
The thickness of the epidermis in +UC group (43.67 μm) was higher than that in -NC (14.33 μm) group at week 10. skin thicknesses of +HLL, +HLH, +HO, +HOHLL and +HOHLH (UVB irradiated) had 28.89, 29.06, 33.17, 28.82, and 27.76 μm, respectively. It was found that HL and HO treatment suppressed increases in epidermal thickness due to the UV irradiation.

Figure 3. Dorsal skin of UVB irradiated hairless mice after supplementation leg bone extracts and applying horse oil.



1)Refer to Table 2

Figure 4. Epidermal thickness (um) of UVB irradiated hairless mice after supplementation of leg bone extracts and applying horse oil



a-cValues of bar with different letter differ significantly at p<0.05.
1)Refer to Table 2

IV. CONCLUSION

In this study we have found that the supplementation of HL and HO spreding were shown to protect skin from UVB-induced wrinkle formation in hairless mice by increasing skin moisture, decreasing TEWL and epidermal thickness. Therefore, these results indicate that supplementation HL and spreding HO at the same time could make synergy to improve skin health and have potential to be as functional cosmetic materials.

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REFERENCES

1. Ali, F. & Sultana, S. (2012). Repeated short-term stress synergizes the ROS signaling through up regulation of NFkB and iNOS expression induced due to combined exposure of trichloroethylene and UVB rays. Molecular and Cellular Biochemistry 360: 133–145.
2. Kim, D., Kim, H. J., Chae, H. S., Park, N. G., Kim, Y. B. & Jang, A. (2014). Anti-oxidation and anti-wrinkling Effects of Jeju horse leg bone hydrolysates. Korean Journal for Food Science of Animal Resources 34: 844-851.
3. Kim, D., Pak, J. I., Chae, H., Kim, Y. B. & Jang, A. R. (2013). Antioxidation effect of leg bone extracts and enzyme hydroltysates from Jeju cross bred horses (Jeju native horse * Thoroughbred). Journal of Life Science 23: 1147-1154
4. Lee, Y. S., Yoon, J. H., Kim, B., Park, C. I., Yoo, W. K., Cho, J. W. & Kim, M. R. (2013). Effects of horse oil on the DNCB-induced contact hypersensitivity in Balb/c mice. The Korea Journal of Herbology 28: 77-81.
5. Matsuura-Hachiya, Y., Arai, K. Y., Ozeki, R., Kikuta, A. & Nishiyama, T. (2013). Angiotensin -converting enzyme inhibitor (enalapril maleate) accelerates recovery of mouse skin from UVB -induced wrinkles. Biochemical and Biophysical Research Communications 442: 38-43.
6. Pyun, H. B., Kim, M., Park, J., Sakai, Y., Numata, N., Shin, J. Y., Shin, H. J., Kim, D. U. & Hwang, J. K. (2012). Effects of collagen tripeptide supplement on photoaging and epidermal skin barrier in UVB-exposed hairless mice. Preventive Nutrition and Food Science 17: 245-253.
7. Quirin, K. W. (2009). Specialty fatty oils for health skin. cosmetic science technology, http://cosmeticsciencetechnology.com/articles/samples/1961.pdf, 08.03.2012
8. Shim, J. S., Kwon, Y. Y. & Hwang, J. K. (2008). The effects of panduratin a isolated from Kaempferia pandurata on the expression of matrix metalloproteinase-1 and type-1 procollagen in human skin fibroblasts. Planta Medica 74: 239–244.
9. Yoo, J. H., Kim, J. K., Yang, H. J. & Park, K. M. (2015). Effects of egg shell membrane hydrolysates on UVB-radiation-induced wrinkle formation in Skh-1 hairless mice. Korean Journal for Food Science of Animal Resources 35: 58-70.