

〈速報〉

Clinical effects of collagen hydrolysates ingestion on UV-induced pigmented spots of human skin: A preliminary study

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ABSTRACT

To examine the effects of ingesting collagen hydrolysates (CHs) from fish scale (fish CH) and swine skin (swine CH) on UV-induced pigmented spots (UV spots) of human skin, a placebo controlled randomized double blind study was conducted. Thirty-nine females ingested 5 g each of control food, fish CH or swine CH daily for 8 weeks. A within-group comparison showed that both fish and swine CHs significantly decreased the area of UV spots between before and 8 weeks after ingestion. In particular, swine CH significantly decreased the area from an early period of 4 weeks after ingestion.

1. INTRODUCTION

Heat-denatured collagen is gelatin, and the collagen hydrolysate (CH), which is formed by the hydrolysis of gelatin by an enzyme is utilized for food products and cosmetics. It has been demonstrated that following oral ingestion of CH, not only amino acids but also di- and tripeptides enter human bloodstream^{1,2)}. In particular, it was reported that large amounts of peptides containing collagen-specific hydroxyproline (Hyp) enter the bloodstream and remain there for a relatively long time³⁻⁵⁾. Zague V. reviewed the effects of CH ingestion on skin properties from a pre-clinical point of view, and pointed out that controlled

clinical trials are needed in addition to the previous pre-clinical and bioavailability assays⁶⁾. A clinical study of the effects of oral ingestion of CH on the skin characteristics showed that ingestion of 10 g of a swine-skin-derived CH (swine CH) for 60 days improved the epidermal water absorption capacity as compared with placebo ingestion⁷⁾. It was also reported that a four-week ingestion of 5 or 10 g of fish-scale-derived CH (fish CH) increased significantly water content in the horny cell layer⁸⁾. Other researchers observed a significantly increased viscoelasticity of human skin after an eight-week ingestion of food containing 4 g of swine CH⁹⁾. To examine the effects of ingesting fish and swine CHs on UV-induced pigmented spots (UV spots) of human skin, a double-blind parallel-group study was conducted.

2. MATERIALS AND METHODS

2.1. Test Food. The following three kinds of test food were used: fish CH²⁾ (Nitta Gelatin Inc., Osaka, Japan), swine CH²⁾ (Nitta Gelatin Inc.), and

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maltodextrin (as control food: placebo, Pinedex TK-16, Matsutani Chemical Industry Co., Ltd., Itami, Hyogo, Japan).

2.2 Study Design and Skin Measurement. A randomized double-blind method was employed with daily ingestion of 5 g of test food for 8 weeks from February to April 2009. This study was performed according to the Helsinki Declaration and was approved by Ethics Committee at Nishi Clinic (Fujiidera, Osaka, Japan) on February 13th 2009. The possible risks of the experiments were explained to all subjects, and informed consent was obtained prior to entry in the study. The subjects were healthy Japanese females aged 35 to 50 years with a subjective symptom of skin roughness or dry skin. Thirty-nine out of 60 subjects who participated in this study were selected on the basis of their medical history, skin condition, and responses to interview questions. They were assigned to the following groups by the randomized double-blind study method. The subjects' mean ages are shown as follows: Fish-CH-fed group: 13 subjects with the mean age of 42.8 ± 3.3 years; Swine-CH-fed group: 13 subjects with the mean age of 42.2 ± 3.9 years; Placebo-fed (maltodextrin) group⁸⁾: 13 subjects with the mean age of 41.8 ± 4.6 years. They cleansed their face as they normally do to remove their makeup, and became acclimated in a room with constant temperature and humidity (temperature, $20^\circ\text{C} \pm 2^\circ\text{C}$; humidity, $50\% \pm 5\%$) for 20 minutes. Then, melanin, pores, porphyrin, hemoglobin, speckles, UV spots, wrinkles and redness were examined using VISIA II (Canfield Imaging Systems, Fairfield, NJ, USA). These measurements were performed prior to the ingestion (0 w), 4 weeks (4 w) and 8 weeks (8 w) after the ingestion. For the purpose of evaluating safety and harmful factors, blood samples were collected from the subjects 0 w and 8 w. The samples were then put to hematological and biochemical tests.

2.3. Statistical Analyses. The parameters

assessed 8 w after the ingestion of CHs were compared with those for the control to calculate *p*-values. Within-group changes 0 w, 4 w and 8 w after the ingestion were compared using the paired *t*-test to calculate *p*-values. The significance of differences was determined using JMP8.0.1 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

VISIA II is a method that detects melanin, pores, porphyrin, hemoglobin, speckles, UV spots, wrinkles and redness on the facial surface by means of image analysis, and evaluates the dimension ratios in proportion to the total measured dimensions as an absolute score. Because initial values for these items differed markedly, it was difficult to carry out a simple comparison between groups 8 w after the ingestion. We hence made comparisons between the changes from 0 w to 4 w and from 0 w to 8 w in each group. For the ingestion of the placebo, no changes were observed in any items assessed. As shown in Table 1 and 2, the area of UV spots decreased significantly between 0 w and 8 w after the ingestion in the fish-CH- and swine-CH-fed groups ($p = 0.034$ and $p = 0.002$, respectively). In particular, the swine-CH-fed group significantly decreased the area from an early period of 4 w after the ingestion ($p = 0.016$) (Table 1 and 2). No abnormalities in blood test results were observed in association with their participation in this study (data not shown), thus substantiating the safety of ingesting the used fish and swine CHs.

In this clinical study, a within-group comparison shows that both fish and swine CHs significantly decrease the area of UV spots 8 w after the ingestion. However, this study has the following study limitation: these three groups each consisting of $n = 13$, were already different groups statistically. Therefore, further study is needed.

An orally-ingested CH is more likely to be

Table 1. Changes in scores of parameters assessed using VISIA II

	Fish CH		
	0 w	4 w	8 w
Melanin	9.01 ± 1.49	8.71 ± 1.47	8.38 ± 1.46
Pores	1.59 ± 1.00	1.52 ± 1.07	1.41 ± 0.87
Porphyrin	0.44 ± 0.85	0.62 ± 1.28	0.27 ± 0.54
Hemoglobin	1.03 ± 0.46	0.99 ± 0.50	1.00 ± 0.48
Speckles	1.89 ± 0.75	1.96 ± 0.82	1.87 ± 0.81
UV spots	4.20 ± 1.93	4.07 ± 1.98	3.71 ± 1.89*
Wrinkles	0.86 ± 0.78	1.04 ± 0.97	0.77 ± 0.77
Redness	1.33 ± 0.90	1.22 ± 0.88	1.12 ± 0.74
	Swine CH		
	0 w	4 w	8 w
Melanin	8.43 ± 2.27	8.27 ± 2.49	8.38 ± 2.13
Pores	1.74 ± 1.12	1.68 ± 1.03	1.53 ± 0.89
Porphyrin	0.30 ± 0.33	0.32 ± 0.37	0.29 ± 0.36
Hemoglobin	1.44 ± 1.02	1.35 ± 0.92	1.57 ± 1.04
Speckles	2.47 ± 1.82	2.55 ± 1.88	2.44 ± 1.82
UV spots	4.39 ± 2.87	3.91 ± 2.60*	3.44 ± 2.38**
Wrinkles	0.58 ± 0.34	0.94 ± 0.53	0.59 ± 0.45
Redness	1.41 ± 0.88	1.58 ± 0.77	1.18 ± 0.74
	Control food		
	0 w	4 w	8 w
Melanin	9.32 ± 1.60	9.23 ± 1.44	9.06 ± 1.57
Pores	1.19 ± 0.56	1.18 ± 0.33	1.19 ± 0.34
Porphyrin	0.23 ± 0.32	0.17 ± 0.21	0.19 ± 0.22
Hemoglobin	1.17 ± 0.63	1.03 ± 0.59	1.11 ± 0.67
Speckles	2.52 ± 1.44	2.11 ± 0.91	2.08 ± 0.94
UV spots	4.95 ± 3.06	4.79 ± 2.42	3.99 ± 2.42
Wrinkles	0.79 ± 0.73	1.11 ± 1.16	0.66 ± 0.51
Redness	1.01 ± 0.53	1.08 ± 0.51	0.94 ± 0.33

Unit is % area. The data are shown as the mean ± SD, n=13 in each group.

Each within-group comparison between before (0 w) and after (4 w and 8 w) the ingestion using paired t-test * p<0.05, **p<0.01

transported to human dermal and epidermal tissues via the peripheral blood vessels in the form of di- and tripeptides after being absorbed into the blood. Proly-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly) are two major components of

them¹⁻⁵). Pro-Hyp was reported to stimulate cell proliferation, cell growth and hyaluronic acid synthesis in cultured dermal fibroblasts^{10, 11}). Hyp-Gly also enhanced the cell growth of mouse primary fibroblasts in a higher extent than Pro-Hyp⁴). These

Table 2. Respective p values according to the corresponding t tests

	Fish CH		Swine CH		Control food	
	0 w vs 4 w	0 w vs 8 w	0 w vs 4 w	0 w vs 8 w	0 w vs 4 w	0 w vs 8 w
Melanin	0.444	0.139	0.447	0.855	0.870	0.669
Pores	0.555	0.124	0.525	0.086	0.914	0.971
Porphyrin	0.438	0.179	0.734	0.794	0.446	0.603
Hemoglobin	0.666	0.755	0.398	0.200	0.459	0.338
Speckles	0.583	0.902	0.213	0.747	0.233	0.241
UV spots	0.538	0.034*	0.016*	0.002**	0.826	0.208
Wrinkles	0.457	0.762	0.070	0.958	0.215	0.453
Redness	0.304	0.092	0.151	0.081	0.525	0.525

In each group, the corresponding t-test was used to compare with before the ingestion; * p<0.05, ** p<0.01.

peptides are suggested to modulate cells and the extracellular matrix proteins of human skin. We therefore believe that these peptides from the CHs used in this study decrease the area of UV spots of human skin via modulating the dermis and probably epidermis. The mechanisms underlying the effects after ingesting used CHs remain to be elucidated.

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