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Effects of oral administration of collagen peptides on skin collagen content and its underlying mechanism using a newly developed low collagen skin mice model

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ABSTRACT

A low collagen skin mice model was established and used to evaluate the effects of orally administered collagen peptide (CP) and glycine, alanine, and proline mixture (GAP) on the collagen content in the skin of mice. This model was established by feeding mice a low protein diet for 8 weeks. The oral administration of 50 mg/kg of CP led to increased collagen content in the skin, although that effect was counteracted in a dose-dependent manner. The oral administration of GAP led to a dose-dependent increase in the collagen content of the mouse skin. Prolyl-hydroxyproline led to a dose-dependent increase in the proliferation of primary cultured murine fibroblasts, and proline caused an increase in fibroblast differentiation. The results of this study demonstrated that CP and GAP influenced the collagen content of mouse skin by changing the state of fibroblasts.

1. Introduction

Skin appearance is a primary indicator of age, with skin becoming unevenly colored, rough, lax, and wrinkled as it ages (Kligman, 1969; Yaar, Eller, & Gilchrest, 2002). Substantial progress has recently been made toward understanding the underlying mechanisms of aging in humans. A feature of aged skin is fragmentation of the dermal collagen matrix. Collagen is a major constituent of the connective tissues in mammals, birds, and fish, and has a unique triple helix configuration with a repeating amino acid sequence of $(\text{glycine–X–Y})_n$, where X and Y are typically proline (Pro) and hydroxyproline (Hyp), respectively (Bos, Rucklidge, Dunbar, & Robins, 1999; Ramshaw, Shah, & Brodsky, 1998). Fragmentation of the collagen matrix typically results from the actions of specific enzymes, such as matrix metalloproteinases, which can impair the structural integrity of the dermis. Fibroblasts that produce and organize the collagen matrix cannot reconnect fragmented collagen, and have consequently evolved to

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regulate the synthesis of collagen and other extracellular matrix proteins in response to mechanical tension. Increased mechanical tension stretches fibroblasts, which causes a coordinated increase in collagen production together with a decrease in collagenase production (Fisher, Varani, & Voorhees, 2008). In aged skin, collapsed fibroblasts produce low levels of collagen and high levels of collagen-degrading enzymes (Fisher, et al., 1997; Fligiel, et al., 2003; Varani et al., 2001, 2006). Therefore, the development of methods capable of accelerating collagen synthesis might become significant treatment strategies for improving the appearance of aged skin.

Collagen peptide (CP) from gelatin hydrolysate is used in several foods and dietary supplements, and the ingestion of CP has been shown to induce numerous biological processes. It was reported that food-derived peptides were detected in human peripheral blood following the ingestion of CP preparations (Iwai et al., 2005). Furthermore, the results of a recent preclinical trial suggest that the daily ingestion of CP can lead to an improvement the skin properties of women during the winter (Matsumoto, Ohara, Itoh, Nakamura, & Takahashi, 2006). The results of *in vitro* experiment, several other animal experiments and preclinical trials have significantly supported the beneficial effects of CP (Matsuda et al., 2006; Moskowitz, 2000; Ngo, Qian, Ryu, Park, & Kim, 2010; Oesser, Adam, Babel, & Seifert, 1999; Tsuruoka, Yamato, Sakai, Yoshitake, & Yonekura, 2007; Wu, Fujioka, Sugimoto, Mu, & Ishimi, 2004). Recently, it was demonstrated that oral ingestion of CP had beneficial effects on dermal matrix synthesis in human clinical trials (Proksch, Schunck, et al., 2014; Proksch, Segger, et al., 2014) and animal experiments (Zague et al., 2011). Prolyl-hydroxyproline (Pro-Hyp) that occurs in human peripheral blood following the ingestion of CP stimulated the growth of fibroblasts in skin, which caused an increase in the number of fibroblasts migrating from the skin (Shigemura et al., 2009). *In vitro* studies using cell culture systems have shown that Pro-Hyp exhibits chemotactic activities toward fibroblasts and peripheral blood neutrophils (Laskin, Kimura, Sakakibara, Riley, & Berg, 1986; Postlethwaite, Seyer, & Kang, 1978), as well as monocytes (Postlethwaite & Kang, 1976). Furthermore, Pro-Hyp was the first edible bioactive peptide derived from collagen hydrolysate to have an effect on chondrocyte differentiation under pathological conditions (Nakatani, Mano, Sampei, Shimizu, & Wada, 2009). Pro-Hyp has also been reported to enhance cell proliferation in cultured human dermal fibroblasts as well as hyaluronic acid synthesis (Ohara et al., 2010). Although a number of studies investigated the effects of adding CP or dipeptides to various cell types, our knowledge about the effect of these agents on the collagen contents of skin or their synthesis in the skin or fibroblasts is poorly understood.

When performing investigations with young, healthy animals, it can be particularly challenging to detect changes in biological reactions following the administration of active materials derived from food, and it is therefore important to develop an animal model capable of confirming the effectiveness of active materials *in vivo*. The total bone mineral content in the femurs of mice fed for 10 weeks on a low protein diet, which was reduced from 14 to 10% total protein, was significantly lower than that in mice administered a normal diet (Koyama et al., 2001). Bone is formed by the deposition of specific minerals at the bone matrix, with collagen accounting for approximately 90% of the components. Taken together, these data indicate a relationship between the mineral and collagen content of bone. Therefore, young mice fed a low protein diet might be useful as an animal model with low collagen content in the skin.

The current study developed a low collagen skin model using hairless mice, which was used to evaluate the effects of orally administered CP and amino acid mixtures on the collagen content of the skin. The influence of dipeptides or amino acids derived from CP on fibroblasts was also examined in primary cultured murine fibroblasts to develop a better understanding of its efficacy.

2. Materials and methods

2.1. *Animals*

Five- and six-week-old male hairless mice (Hos:HR-1) were purchased from Japan SLC (Shizuoka, Japan), housed under controlled conditions (ambient temperature, 23 ± 2 °C; relative humidity, 60 ± 10%; 12-h light/dark cycle), and given commercial pellets (AIN-93M, Oriental Yeast, Tokyo, Japan) and water *ad libitum*. All procedures regarding animal care and use were carried out in accordance with the regulations dictated by the Experimental Animal Care and Use Committee of Fukuoka University.

2.2. *Chemicals*

A low protein diet, where the protein (milk casein) content of AIN-93M was reduced from 14 to 10%, was purchased from Oriental Yeast. For *in vivo* experiments, collagen peptide (CP, Nippi peptide FCP, MW 5000 Da, glycine:alanine:proline (Pro):hydroxyproline (Hyp):others = 3:1:1:1:4, approximately, molar ratio in CP) was obtained from Nippi (Tokyo, Japan), whereas Pro, alanine, and glycine were from Kyowa Hakko Bio (Tokyo, Japan). For the *in vitro* experiment, Hyp and Pro were purchased from Nacalai Tesque (Kyoto, Japan), whereas prolyl-hydroxyproline (Pro-Hyp) was purchased from Bachem (Bubendorf, Germany). All other reagents used in this study were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.3. *Effects of low protein diet on skin collagen contents*

Nine-week-old mice were randomly divided into groups (n = 10). The normal diet group was fed a normal diet (AIN-93M), whereas other groups were fed a low protein diet for 4, 8, or 12 weeks. The total food intake for each group was recorded five times a week and the body weight of each mouse was recorded once a week. Before the experiment (0 week) and after 4, 8, and 12 weeks of consuming the diet, the mice were euthanized without suffering by cervical dislocation and samples of dorsal skin were removed for measurement of the Hyp content. Skin samples (disk diameter 8 mm) were stored at –30 °C prior to being analyzed.

2.4. *Influence of CP and glycine, alanine, and proline mixture on collagen levels in a low collagen skin mice model*

Nine-week-old mice were randomly divided into groups (n = 9 or 10). The normal diet group was fed a normal diet (AIN-93M),