Chapter 5 Ingredients of Foods for Elderly Persons

1.3 Collagen peptides

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1. Introduction

In today's super aging society, more and more elderly persons are seen to require long-term hospitalization or home healthcare due to illness or injury. When bedridden, such elderly persons have difficulty changing their posture by themselves, and are prone to pressure ulcers if not receiving sufficient nutrition.

The purpose of most conventional pressure ulcer treatments is to prevent the ulcers from becoming chronic by cleaning and protecting the affected areas, as well as maintaining the area moist. In addition, the common method of promoting the healing of pressure ulcers is to ensure proper nutritional management and feed the elderly with nutrition, which is partially composed of dietary supplements. For instance, the amount of essential amino acids is often discussed for protein support from the nutritional viewpoint.

Collagen peptide (CP) discussed in this chapter was developed in hope that it would contribute to improving pressure ulcers as a functional ingredient, rather than to feed amino acids. Sections 2 to 4 describe the latest information on the functional material CP, focusing on its absorption kinetics after oral intake, effects on pressure ulcers, and its mechanism. Section 5 introduces applications to enteral feeding products based on ideal amino acid scores.

2. CP absorption

It has already been reported that CP-derived dipeptides and tripeptides such as hydroxyproline (Hyp) are detected in human blood after CP is orally ingested[1) 2).] This chapter discusses CP supplied by Nitta Gelatin Inc. (Collapep PU). The pig hide-derived CP is characterized by an average molecular weight of 1300 and containing dipeptides such as prolyl-hydroxyproline (Pro-Hyp) and hydroxyprolyl-glycine (Hyp-Gly). To elucidate the kinetics of peptides entering into the blood after CP intake, absorption tests were conducted in 5 male volunteers from Nitta Gelatin. After having them intake 8 g of CP, their blood was sampled from the vein at 0, 0.5, 1, 2, and 4 h to measure Pro-Hyp and Hyp-Gly, peptides usually found in the collagen alignment, and prolyl-hydroxyprolyl-glycine (Pro-Hyp-Gly) and grycil-prolyl-hydroxyproline (Gly-Pro-Hyp), both of which are tripeptides. The results found that Pro-Hyp transferred to the blood the most, followed by Hyp-Gly[3)]. As for the absorption behavior of Pro-Hyp, the maximum blood concentration was reached at about 1 h, after which the concentration declined gradually (Figure 1). Furthermore, a rat

experiment revealed that Pro-Hyp in the bloodstream reached the dermis at about 30 min[4].

Meanwhile, the physiological functions of Pro-Hyp on cells include chemotaxis by which fibroblast cells gather[5], promotion of cell multiplication[6], and promotion of hyaluronic acid production[7]. Hyp-Gly has been demonstrated to significantly enhance the multiplication of the first-generation fibroblasts cultured on collagen gel compared to Pro-Hyp[8]. In this way, Pro-Hyp and Hyp-Gly absorbed after CP intake are believed to repair damaged skin tissues and promote remodeling.

Oral administration of CP is also expected to demonstrate strong effects on the treatment of pressure ulcers, discussed in the following section, through the above absorption and transfer processes after CP intake.

3. Effects of Collapep PU intake on pressure ulcers

This section discusses the results of clinical studies investigating the efficacy of Collapep PU in pressure ulcer patients. One study found the promotion of wound healing by combining CP and amino acids mixtures[9], suggesting the efficacy of CP intake for pressure ulcer patients. However, the efficacy of the single CP intake has never been reviewed, and therefore, this section aims to validate the effects of single CP use.

Collapep PU was used as the study food and maltodextrin as the placebo (TK-16, Matsutani Chemical Industry Co., Ltd.). Subjects were 81 Indian male and female patients with Stage-2 and -3 pressure ulcers. They were randomized into treatments with Collapep PU (CP group, 40 subjects) and placebo (placebo group, 41subjects) in a double-blind study. The patient age ranged from 18 to 70 years, and their BMI was ≥18.5 but <35.0. People who were diabetic, pregnant, or breastfeeding were excluded from registration.

The study food and placebo were administered daily at a dose of 5 g morning and evening (10 g/day) for 16 weeks, and evaluated using the three following international assessment scales:

(1) PUSH (Pressure Ulcer Scale for Healing) score (0-17 points: the worse the symptom is, the higher the score is)
(2) PSST (Pressure Score Status Tool) score (13-65 points: the worse the symptom is, the higher the score is)
(3) Wound area on photo (cm^2)

The difference in the above scores between baseline and 16 weeks was evaluated. The subjects were placed on standard treatment while being given the study food as an additional treatment. To confirm their nutritional state, changes in serum albumin and blood total protein levels were used as indicators. This study was conducted in accordance with the Helsinki Declaration whereby written informed consent was obtained from each subject prior to the start of the study.

No adverse events were observed in both groups. The results of subjects excluding those who

dropped out of the study were statistically compared by the two-way analysis of variance (two-way ANOVA). The results were as follows:

(1) The PUSH score as -5.89 ± 1.97 in the CP group and -2.67 ± 1.26 in the placebo group, with a statistically significant intergroup difference ($p<0.0001$).

(2) The PSST score was -10.49 ± 3.79 in the CP group and -6.41 ± 3.63 in the placebo group, with a statistically significant intergroup difference ($p<0.0001$) (Table 1).

(3) The wound area was -10.04 ± 8.64 in the CP group and -7.85 ± 7.63 in the placebo group, with a statistically significant intergroup difference ($p<0.0001$) (Table 2).

Blood chemistry analyses showed that both blood protein and serum albumin levels were within the normal range in all subjects during the study period, suggesting their good nutritional condition (Table 3).

There was also no statistically significant difference between the two groups in terms of BMI, gender, and age. Compared to the placebo group, significant improvement was found in the CP group in scores on the three evaluation scales, suggesting that the addition of Collapep PU intake to the standard treatment promotes healing pressure ulcers.

4. Mechanism promoting wound healing by Collapep PU

4.1 Mechanism promoting wound healing by oral administration of Collapep PU

The efficacy of oral administration of CP for the treatment of pressure ulcers is broadly defined as its effects on healing wounds. The following paragraphs discuss the effects of Collapep PU on a rat model with skin wounds.

Using model rats with skin wounds, Horiuchi *et al.* compared the wound healing promotion effects between Collapep PU and milk casein (control food, casein group), administered at 1 g daily[10]. In the initial stage of wound healing, the production of matrix metalloproteinase (MMP), which breaks down unwanted substrates due to the injury, increases. At day 0 of recovery, MMP-9 production of the Collapep PU group was significantly increased compared to the milk casein group. The analyses of localization in the skin tissue revealed that MMP-9 production was expressed locally near the basement membrane at the boundary between the epidermis and dermis of the wound area in the Collapep PU group, while in the casein group, it was found dispersed all over the skin. On day 4 of recovery, MMP-9 production had stopped in both groups.

The percentage of nascent collagen (NaCl-soluble collagen) in the skin tissue during the recovery process was also measured. A significant increase in nascent collagen was found in the Collapep PU group compared to the casein group from at day 1 of recovery[11]. The results suggest that oral administration of Collapep PU promotes the efficient break down of unwanted substrates by locally promoting MMP-9 production near the wound, thereby producing nascent collagen. Finally, it was concluded that the oral administration of Collapep PU promotes the remodeling and

re-epithelization of skin tissue.

4.2 Mechanism of pressure ulcer healing actions of Pro-Hyp

What signals do specific CP-derived peptides, such as Pro-Hyp, send to fibroblasts? In what mechanism are such peptides involved in the promotion of wound healing?

We focused on Pro-Hyp found in Collapep PU, and investigated *in vitro* its effects on the genetic expression of cells. Three-dimensional culture with normal human dermal fibroblasts (NHDF [NB]) (Kurabo Industries Ltd.) embedded in floating collagen gel was used for analysis. The cells were embedded in the gel at a concentration of 5×10^5 cells/ml × 500 ul, and Pro-Hyp was added to the culture media to bring the final concentration to 5 mM. Twenty-four hours after cultivation, the collagen gel was broken down using collagenase, and the cells were sampled. Total RNA samples were collected from the cells, and the genetic expression level was quantified using the real-time polymerase chain reaction (PCR) method.

The results suggested that Pro-Hyp increases the mRNA expression of hyaluronan synthase 2 (*HAS2*), signal transducer and activator of transcription 3 (*STAT3*), hyaluronic acid receptor (*CD44*), *MMP-9* (gelatinase B), and *MMP-1* (interstitial collagenase), whereas it inhibits the mRNA expression of receptor for hyaluronic acid mediated mobility (*RHAMM*) and type I collagen (*col1a1*). These findings suggest that Pro-Hyp increases the mRNA expression of *HAS2* via the upstream gene of *STAT3* (Figure 2). Given that the mRNA expression of hyaluronic acid receptor *CD44* increases as well, a more optimum microenvironment are expected to be created for fibroblasts. Furthermore, an increased mRNA expression of *MMP-9,-1* matches the transient increase in MMP-9 production at the protein level in the initial stage of wound healing, described in the previous section.

These findings suggest that Pro-Hyp functions as a signal on cells in each process of wound healing and plays the role of adjusting genetic expression related to the remodeling of damaged skin tissue.

5. Applications of Collapep PU to enteral feeding products

Protein contained in enteral feeding products need to have high nutritional value. However, Collapep PU contains a very small amount of essential amino acids, and no tryptophan in particular. In the preparation or designing of collagen peptide-based enteral feeding products, it is hence ideal to combine them with protein with high nutritional value. The following paragraph discusses the combination of casein sodium, a protein often used in enteral feeding products, from the viewpoint of amino acid score.

Table 4 shows the amino acid composition of different types of protein. If Collapep PU and casein sodium are combined, compositions were converted into relative figures per 1 g of nitrogen as the nitrogen-to-protein conversion factor differs between the two products. The amino acid pattern

indicates the ideal essential amino acid levels in adults. If these values are achieved, it means that the amino acid score is calculated as 100. To maintain 70 mg of tryptophan, if Collapep PU and casein sodium are combined at a ratio of 1:4, the amino acid score of 100 can be established. The recommended protein level of 60 g/day for males over 70 years of age in the Japanese food intake standards is premised on the intake of protein with high nutritional value such as casein sodium. If 12 g out of the 60 g is replaced by Collapep PU, the amino acid score of 100 can be maintained. This means that consuming 12 g/day fulfills the required level of 10 g/day for providing the physiological functions of Collapep PU, discussed in section 3, enabling the design of an enteral feeding product meeting both objectives of promoting pressure ulcer healing and amino acid supply.

6. Conclusion

The findings of this study suggest that CP-derived peptide molecules such as Pro-Hyp with physiological functions act directly on skin cells and genetically adjust the remodeling of damaged skin tissues during the wound healing process. In addition, the intake of Collapep PU containing these molecules was found to demonstrate healing promotion effects even in patients with pressure ulcer and other symptoms for which the efficacy of the original treatment is reduced.

As an application for, If combined with substances with high nutritional value such as casein sodium, Collapep PU has been demonstrated to be used as an enteral feeding product while properly supporting originally intended nutrients. In the future, further study will be necessary to elucidate the mechanism of CP promoting pressure ulcer healing, and their applications will be required for the development of various CP-based food products for the elderly.

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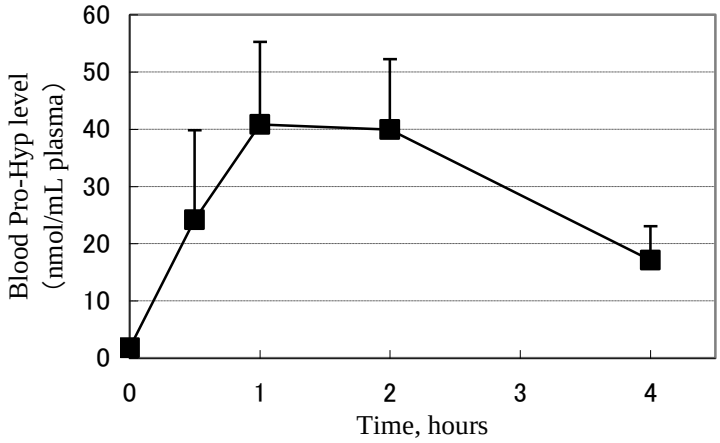

Figure 1. Absorption behavior of Pro-Hyp in human venous blood

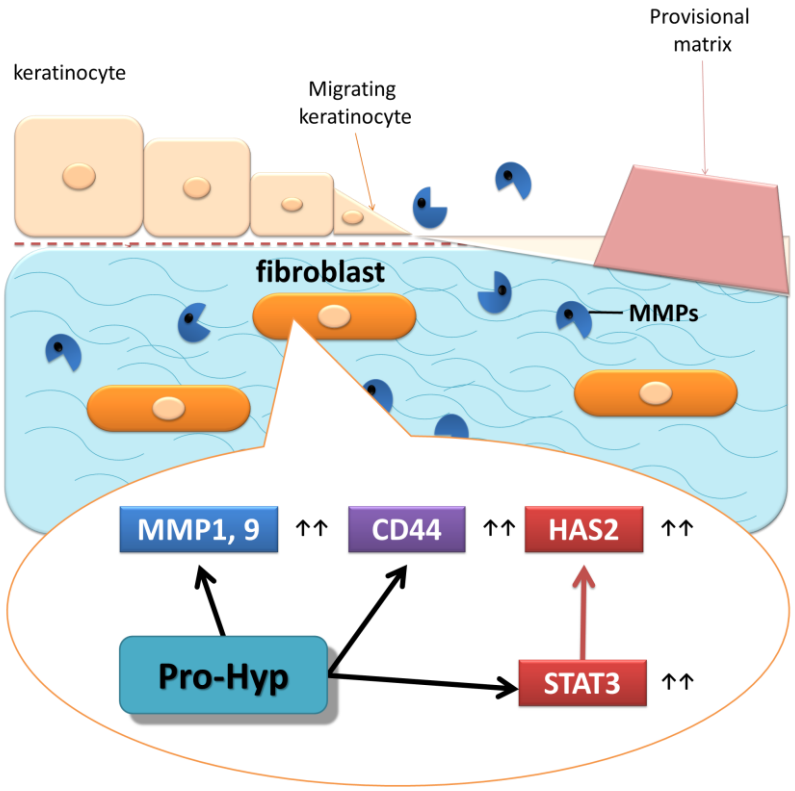

Figure 2. Mechanism of wound healing

Table 1 Changes in PSST score

	Baseline	16 weeks	Improvement rate	
CP group	30.20 ± 1.69	19.71 ± 3.08	-10.49 ± 3.79	P<0.0001
Placebo group	29.79 ± 2.31	23.38 ± 3.85	-6.41 ± 3.63	

P-value: Two-way ANOVA (improvement rate in CP group vs. placebo group)

Improvement rate = (Score at 16 weeks) – (Score at baseline)

Table 2 Changes in wound area

	Baseline	16 weeks	Improvement rate	
CP group	13.23 ± 9.56	3.19 ± 2.88	-10.04 ± 8.64	P<0.0001
Placebo group	12.85 ± 10.29	5.00 ± 3.88	-7.85 ± 7.63	

(in cm^2)

P-value: Two-way ANOVA (improvement rate in CP group vs. placebo group)

Improvement rate = (Area at 16 weeks) – (Area at baseline)

Table 3 Changes in blood albumin and total protein levels

Albumin (g/L)	Baseline	16 weeks
CP group	42.05 ± 5.95	45.06 ± 7.46
Placebo group	41.87 ± 6.44	42.97 ± 5.51

Total protein (g/L)	Baseline	16 weeks
CP group	72.36 ± 5.56	74.48 ± 8.86
Placebo group	72.00 ± 4.25	73.58 ± 6.59

Table 4　Amino acid composition

	Amino acid Pattern (mg)	Amino acid composition		
		Collapep PU	Casein sodium	Combination
Histidine	120	39	200	168
Isoleucine	180	67	363	304
Leucine	410	161	631	538
Lysine	360	239	519	463
Methionine + Cystine	160	50	213	180
Phenylalanine + Tyrosine	390	161	700	593
Threonine	210	94	288	249
Tryptophan	70	0	88	70
Valine	220	144	463	181
Amino acid score		0	100	100