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Original article

Anti-atherosclerotic and anti-glycation effects of collagen peptides

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Abstract

Collagen peptides derived from fish scales and pig skin have a molecular weight of about 1,000, which is a low molecular collagen peptide (CP). We have conducted two RCTs and reported that CP has anti-atherosclerotic effects and improves glucose tolerance. However, the recent finding that CP activates fibroblasts has opened the door to discussing the mechanism of action of CP from a new perspective. This paper introduces the involvement of glycative stress in the mechanism of action of CP. In the first RCT, pulse wave velocity (baPWV) was shown to improve. The results of multivariate analysis of factors involved in the improvement of baPWV indicated that the involvement of improvement in arterial patency, independent of the effect on blood pressure improvement, was important. The 2nd RCT showed that CP intake improved insulin resistance and reduced cutaneous autofluorescence (SAF), a measure of terminal glycation products (AGEs) accumulation in the skin including toxic AGEs (TAGEs), which are highly toxic. One possible mechanism of action is the fibroblast activating and proliferative effects of the CP-derived dipeptides Pro-Hyp (PO) and Hyp-Gly (OG). The production of matrix proteins by fibroblasts is important for maintaining vascular wall homeostasis. In particular, elastic fibers (elastin) are strongly involved in the elasticity of arteries. The second mechanism is aldehyde trapping by CP-derived dipeptides and amino acids. Among the many short-chain aldehydes induced by postprandial hyperglycemia (blood glucose spikes), glyceraldehyde is the most dangerous, generating TAGE. Aldehydes also adversely affect insulin biosynthesis in pancreatic beta cells because they readily cross cell membranes. From the above, it can be inferred that aldehyde trapping is of great significance. In conclusion, since CP has anti-atherosclerotic and anti-glycation effects, it is expected to contribute to the extension of healthy life span through the prevention of cerebro-cardiovascular diseases.

KEY WORDS: collagen peptide, pulse wave propagation velocity, anti-atherosclerotic effect, advanced glycation endproducts (AGEs), AGE Reader

Introduction

Collagen constitutes about 30% of the proteins in our bodies. Collagen is characterized by its triple-helical, fibrous form and its molecular weight of approximately 300,000, making it a very large protein. Since it is said that the human body is composed of about 20% protein, it is calculated that a person weighing 50 kg has 10 kg of protein, which contains about 3 kg of collagen. When collagen is heated and dissolved, the triple-helical structure is loosened to form gelatin, and when gelatin is further hydrolyzed using special enzymes, its molecular weight is further reduced, and it is generally called collagen peptide. The molecular weight of collagen peptides derived from fish scales and pig skin, which have recently been used in research, is about 1000, and these are called low molecular collagen peptides (CP). It is also easily absorbed. It has been shown that orally ingested CP is transferred into the blood, not only as an amino acid but also as a dipeptide or tripeptide, which is a combination of two or three amino acids, and that it remains in the blood for a relatively long time [1]. CP has been reported to contain high concentrations of dipeptides such as Pro-Hyp and Hyp-Gly. In the field of cosmetic dermatology, they have been reported to be highly effective for skin beautification. Recent studies have also reported that CP supplements have a significant effect on the reduction of "blemishes," which have a significant impact on the "appearance" of the skin after one month [2]. Our laboratory has conducted three RCTs with CP and reported its anti-atherosclerotic effects and improvement of skin conditions [3-5]. In this paper, we introduce the novel functional properties of CP that support these clinical results from the viewpoints of fibroblast activation and glycative stress.

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Results and Discussion

Anti-atherosclerotic effects of CP

Former RCT on the anti-arteriosclerotic effect of CP, chicken-derived CP was administered to 58 normotensive to mildly hypertensive subjects (mean age 52.8 ± 8.6 years, 30 males)[3].

The results showed that, over the 12-week study period, arterial progressivity, one of the indicators of arterial stiffness as measured by pulse wave velocity of propagation (baPWV) testing, was significantly improved. An important finding of this study is that there was a significant reduction in systolic blood pressure and a trend toward improvement in serum NOx, one of the indicators of vascular endothelial function, only in the CP group after 12 weeks compared to baseline ($p < 0.1$). In other words, the angiotensin-converting enzyme inhibitory effect of chicken-derived CP, which has been previously reported in basic research, and the accompanying increase in NO via an increase in bradykinin[6], may have been proven in human clinical trials. Tomosugi et al.[7] also reported a similar single-arm study in which 30 subjects (mean age 53.7 ± 7.2 years, 15 males) were treated with CP derived from porcine skin for 24 weeks and found that it improved arterial patency. The index used in this study was the Cardio Ankle Vascular Index (CAVI)[8], which is less sensitive to blood pressure than the aforementioned baPWV. In this study, CAVI improved compared to baseline, and there was a significant reduction in the L/H ratio in the high-risk group for atherosclerosis, where the initial value of the ratio of low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) (L/H ratio) was greater than 2.5 ($p = 0.025$).

In the first RCT, the CP administration test was conducted on 70 examinees (mean age 72 ± 8 years, 18 males) at the "Anti-Aging Dock" to evaluate arterial stiffness[4]. The subjects were randomly assigned to either (A) the actual drug group: 2.5 g of CP (high dipeptide content, Pro-Hyp and Hyp-Gly > 3000 ppm) derived from pork skin, or (B) the placebo group: 2.5 g of whey protein, using a random number table, and 36 in the placebo (P) group. During the observation period, one participant dropped out of the study after experiencing a mild diarrhea adverse event (which resolved quickly after participation in the study was discontinued). Another participant was lost to follow-up due to relocation, and no data were available. An additional four participants dropped out of the study due to taste intolerance and refusal of sustained intake. Data from 64 subjects (30 in the CP group and 34 in the P group) were available for the final analysis. Blood pressure measurements and baPWV tests were performed on the subjects before and after administration, in addition to general blood sampling, to determine differences between the two groups.

The results showed that neither the CP nor the placebo group had side effects that caused problems with blood tests (***Table 1***). As shown in ***Table 2***, baPWV at 12 weeks in the CP group (30 subjects, 6 males) was significantly lower than baseline data ($p = < 0.01$). In contrast, baPWV at 12 weeks in the placebo group (34 subjects, 8 males) was not significantly different from baseline values. There was no significant difference between the two groups with respect to systolic blood pressure. baPWV improvement rate related factors were examined by multiple regression analysis, and the results showed that being in the actual drug group was selected as a significant explanatory factor independent of age, gender, systolic blood pressure before starting, and percentage change in systolic blood pressure, suggesting that pork skin-derived CP has a function that resembles chicken-derived CP and that it has an ameliorative effect on arterial patency independent of its blood pressure ameliorative effect (***Table 3***).

One of the reasons why CP has an arteriosclerosis ameliorative effect independent of its blood pressure ameliorative effect may be the effects of CP-derived dipeptides, Pro-Hyp (PO) and Hyp-Gly (OG), on fibroblasts. The CPs used in the aforementioned studies all contain large amounts of PO and OG. The PO and OG have been reported to promote fibroblast proliferation[9,10]. Elastic fiber (elastin) is strongly involved in the elasticity of arteries, and it is

Table 1. Blood examination in the first RCT on CP.

		CP (n = 30)		Placebo (n = 34)	
		Base line	12 weeks	Base line	12 weeks
sCr	mg/dL	0.76 ± 0.20	0.78 ± 0.19	0.77 ± 0.16	0.79 ± 0.18
BUN	mg/dL	15.1 ± 4.0	14.9 ± 4.1	16.5 ± 4.3	16.5 ± 4.6
eGFR	mL/min/1.73 m^2	66.8 ± 16.3	64.2 ± 14.3	63.0 ± 10.4	61.6 ± 11.8
LDL-C	mg/dL	98 ± 50	101 ± 47	107 ± 35	105 ± 27
HDL-C	mg/dL	65 ± 12	65 ± 11	59 ± 11	59 ± 13
TG	mg/dL	113 ± 63	102 ± 49	112 ± 55	108 ± 53
GOT	mg/dL	25 ± 6	22 ± 4	23 ± 6	25 ± 9
GPT	mg/dL	22 ± 9	20 ± 7	21 ± 10	23 ± 16
γ-GTP	cm/s	34 ± 35	31 ± 32	27 ± 17	28 ± 19
Hb	g/dL	13.6 ± 1.0	13.5 ± 1.1	13.5 ± 1.3	13.8 ± 1.4
Ht	%	41.1 ± 2.5	41.0 ± 3.1	40.7 ± 3.6	41.0 ± 3.9

Values are expressed as the mean ± standard deviation. sCr, serum creatinine; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; γ-GTP, γ-glutamyl trans peptidase; Hb hemoglobin; Ht, hematocrit value.

Table 2. BP, pulse rate and baPWV in the first RCT on CP.

	Base line	12 weeks	Change ratio (%)
Systolic BP (mmHg)			
CP	129 ± 15	125 ± 16	−3.1 ± 9.6
Placebo	130 ± 17	131 ± 17	1.1 ± 11.0
Diastolic BP (mmHg)			
CP	74 ± 10	72 ± 11	−2.4 ± 11.7
Placebo	76 ± 11	76 ± 11	0.6 ± 11.9
Pulse rate (bpm)			
CP	69 ± 7	69 ± 10	0.3 ± 11.8
Placebo	70 ± 8	71 ± 9	1.3 ± 7.8
baPWV (cm/s)			
CP	1709 ± 248	1611 ± 221	−5.4 ± 6.5 *
Placebo	1670 ± 266	1646 ± 211	−0.7 ± 8.7

Values are expressed as the mean ± standard deviation. CP group, n = 30; Placebo group, n = 34, *p <0.01, comparison between groups. BP, blood pressure; baPWV, brachial-ankle pulse wave velocity; CP, collagen peptide.

Table 3. Correlation analysis in the first RCT on CP (n = 64).

	Univariate		Multivariate	
	r	P	β	P
Age	−0.048	0.707		
Sex (male)	0.099	0.437		
Systolic BP (base line)	−0.166	0.191		
baPWV (base line)	−0.462	<0.001	−0.428	<0.001
baPWV (12 weeks)	0.071	0.578		
CP ingestion (+)	5.682	0.007	0.279	0.013

BP, blood pressure; baPWV, brachial-ankle pulse wave velocity; CP, collagen peptide.

possible that the proliferative effect of fibroblasts induced to proliferate by PO and OG also contributes to elastin synthesis, thereby improving arterial elasticity.

Another possibility is the action of CP-derived dipeptides and amino acids to trap blood aldehydes. Although 99% of the glucose in the blood presents a saccharide form with a cyclic structure, some of it presents an open ring structure with aldehyde groups exposed. During hyperglycemia, especially postprandial hyperglycemia (glucose spike), the aldehyde-type glucose triggers the formation of short-chain aldehydes such as 3-deoxyglucosone, glyoxal, methylglyoxal and glyceraldehyde [11]. Aldehydes readily cross cell membranes and induce carbonylative modifications of peptides during insulin biosynthesis in pancreatic beta cells, resulting in the generation of glycated insulin and increased insulin resistance [12]. These aldehydes are highly reactive and easily injure vascular endothelial cells, resulting in an increased susceptibility to induce thrombus formation and an increased risk of cardiovascular events. These aldehyde traps are attracting attention as a strategy to protect the vascular wall from injury.

Anti-glycation effects of CP

Fish-derived CP has been reported to improve fasting blood glucose, HbA1c levels, and insulin resistance index (HOMA-R) in patients with type 2 diabetes [13, 14].

As mentioned earlier, postprandial hyperglycemia (glucose spike) produces carbohydrate-derived aldehydes, which react with proteins to cause carbonylative modifications and undergo several additional reactions to produce advanced glycation endproducts (AGEs). Aldehydes readily cross cellular and nuclear membranes, resulting in the formation and accumulation of AGEs in blood vessels, tissues, and cells. Aldehydes also promote damage not only to proteins but also to lipids and nucleotides in the body. Since intracellular AGEs induce ER stress and impair cellular functions, inhibition of AGE formation may be a new molecular target for organ protection in diabetes with respect to prevention of aging-related and chronic diseases [15].

To date, it has not been fully clarified whether CP affects AGEs. The second RCT aimed to confirm the efficacy of daily CP intake on AGE levels in skin and subcutaneous blood vessels and on glucose metabolism in blood [5]. A total of 30 subjects (male 8, female 22), aged 47-87 (69 ± 11) years without overt diabetes, were randomly assigned to the test food or placebo for 12 weeks. The test food was 5g of fish-derived CP (TYPE-S, average molecular weight = approximately 500-1200 Da, containing 3 g/kg of Pro-Hyp and Hyp-Gly; Nitta Gelatin Co., Osaka, Japan) and the placebo was maltodextrin (Paindex # 2 AG; Matsutani Chemical Industry Co, Hyogo, Japan). AGE levels were

measured at the beginning and end of the study; AGEs were evaluated from skin via skin autofluorescence using an AGE reader (Diagno Optics Technologies BV, Groningen, Netherlands). The results showed significantly lower AGE levels in the CP group compared to the placebo group (*Fig. 1*) and a lower insulin resistance index; HOMA-R = fasting insulin level (μU/mL) x fasting blood glucose level (mg/dL) / 405). Furthermore, a strong positive correlation was observed between AGEs and the rate of change in HOMA-R levels in both groups (*Fig. 2*). These results suggest that fish-derived CP may reduce AGE levels and improve insulin resistance. The aforementioned report by Tomosugi et al.[7] also measured toxic AGEs (TAGEs) derived from glyceraldehyde, a sugar metabolism intermediate, which is particularly toxic among AGEs in conjunction with the anti-atherosclerotic effect of CP. A significant decrease in TAGE was observed in all subjects ($p = 0.031$). One of the reasons for the reduced TAGE formation may involve glyceraldehyde trapping by CP-derived dipeptides and amino acids.

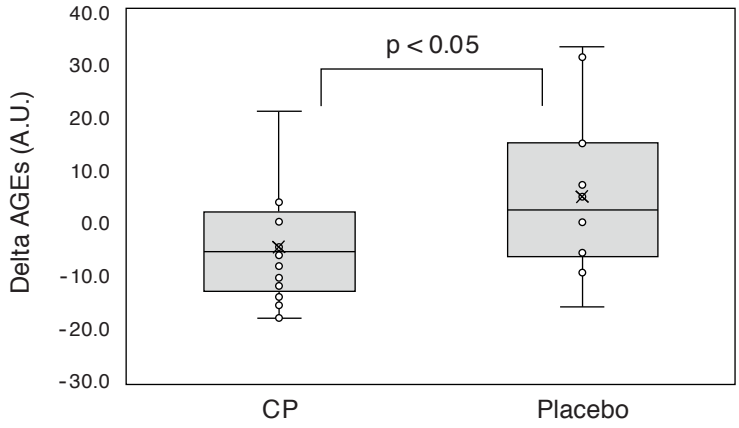

Fig. 1. **Comparison of percent change in AGEs in each group.**

CP group, n = 16; Placebo group, n = 14. The two groups were compared by one-way ANOVA. AGEs: advanced glycation endproducts, evaluated by using AGE Reader; CP: collagen peptides.

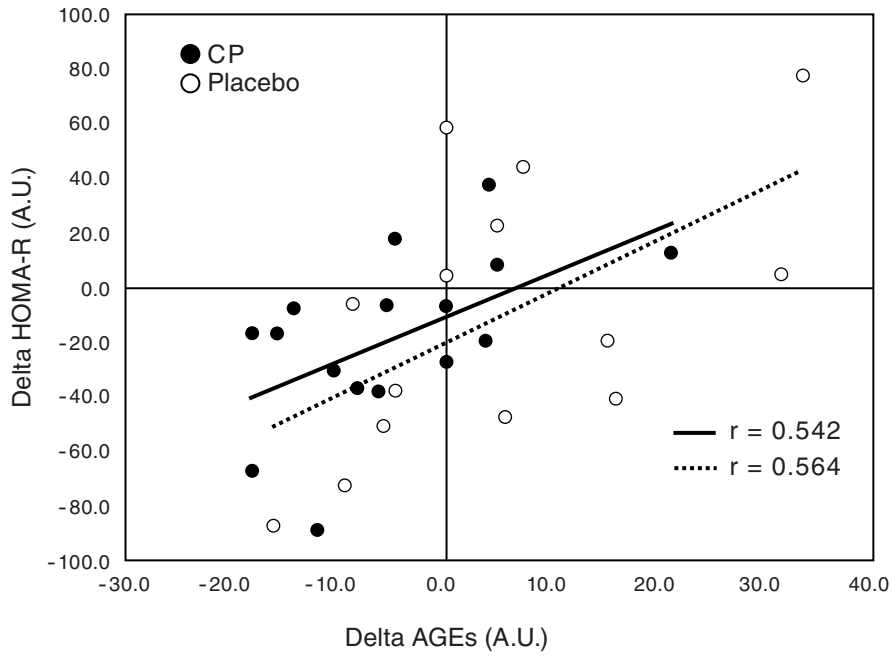

Fig. 2. **Correlation between percent change in AGEs and percent change in HOMA-R in the collagen peptide (CP) and placebo (P) groups.**

Black circles indicate the CP group (n = 16) and white circles the placebo group (n = 14). Spearman's rank correlation coefficients with the solid line indicating the CP group (r = 0.542) and the dotted line indicating the placebo group (r = 0.564). Both groups showed a strong correlation between the rate of change in AGEs and the rate of change in HOMA-R. HOMA-R: homeostasis model assessment ratio; AGEs: advanced glycation endproducts, evaluated by using AGE Reader; CP: collagen peptide.

Conclusion

In this paper, we introduced the possibility that fibroblast activation and antiglycation are involved in the mechanism of the anti-atherosclerotic effect of CP intake. The detailed mechanism of the anti-glycation effect, in particular, is still unclear in many respects. For example, the variability in the data suggests that there are individual differences in the production of AGEs. This may be due to individual differences in the degree of carbohydrate-derived aldehyde production even when blood glucose spikes of the same magnitude occur. The trapping effect of CP-derived amino acids is expected to be particularly effective for those with high levels of aldehyde production. AGEs, which were evaluated as an anti-glycation agent, are found to increase not only in hyperglycemia-induced glycative stress but also in dyslipidemia as fatty acid-derived aldehydes including methylglyoxal, as described earlier [16]. The formation of short-chain aldehydes by fatty acid oxidation may reaffirm the significance of antioxidants, and it will be important to answer these questions as we search for additional benefits from CP intake.

As Japan's population ages, the extension of healthy life expectancy is a major theme. The anti-arteriosclerosis and anti-glycation effects of CP are expected to contribute to the extension of healthy life expectancy through the prevention of cerebrovascular diseases. In the future, the intake of CP as a food may become one of the options for extending healthy life span. Currently, CP is generally consumed dissolved in tea, coffee, black tea, etc. In the future, it will also be necessary to search for the optimal combination to maximize the functional effects of CP.

Declaration of Conflict of Interest

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