**Note**

**Chicken Collagen Hydrolysate Reduces Proinflammatory Cytokine Production in C57BL/6.KOR-ApoE<sup>shl</sup> Mice**

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**Summary**

Effects of chicken collagen hydrolysate (CCH) on atherogenesis in apolipoprotein E-deficient C57BL/6.KOR-ApoE<sup>shl</sup> mice were investigated in this paper. The mice were fed on either a normal CE-2 diet (control group) or a diet containing 10% CCH (CCH group) for 12 wk. Compared with that of the control, the amount of total plasma cholesterol, total hepatic cholesterol and hepatic triglycerides in the CCH group was reduced by 14.4, 24.7 and 42.8%, respectively. Histological analysis results showed that the abundance of diffuse hepatic lipid droplets and fat vacuoles was decreased in the CCH group. Meanwhile, the concentration of proinflammatory cytokines in the CCH group plasma, including interleukin-6 (IL-6), soluble intercellular adhesion molecule-1 (sICAM-1), and tumor necrosis factor-α (TNF-α), was downregulated by 43.4, 17.9 and 24.1%, respectively. The present results suggested that CCH treatment might help prevent atherosclerosis through not only its lipid-lowering effects but also inhibiting expression of inflammatory cytokines.

**Key Words**

chicken collagen hydrolysate, proinflammatory cytokine, lipid, atherosclerosis

In recent years, inflammation has emerged as a key factor in the development of atherosclerosis. Atherosclerosis, formerly considered a lipid storage disease, actually involves an ongoing inflammatory response (1). A substantial number of studies have established that excessive inflammation within the arterial wall is a risk factor for cardiovascular disease and promotes atherogenesis (2, 3). Therefore, factors that act to limit inflammation in this setting may prove to be beneficial in reducing disease progression.

Chicken extract is widely used in foods all over the world. Chicken collagen hydrolysate (CCH) is extracted from chicken legs and contains abundant peptides with molecular weights less than 6,000 Da. CCH has strong angiotensin I converting enzyme (ACE)-inhibitory activity and antihypertensive effects in spontaneously hypertensive rats (4). The antihypertensive effect of CCH has also been confirmed in mildly hypertensive human subjects (5). We have also reported that CCH prevents hypertension and cardiovascular disorders induced by N-nitro-L-arginine methyl ester (L-NAME) in Wistar-Kyoto rats (6).

In this study, we examined whether dietary administration of CCH affected the expression of proinflammatory cytokines and retarded atherogenesis in C57BL/6.KOR-ApoE<sup>shl</sup> mice.

**Materials and Methods**

CCH was obtained from chicken legs by the method described by Saiga et al. (4).

Five-week-old male C57BL/6.KOR-ApoE<sup>shl</sup> mice were purchased from Japan SLC, Inc. (Tokyo, Japan) and fed on a CE-2 diet (CLEA Japan, Inc., Tokyo, Japan) and water ad libitum in an environmentally controlled room (23°C, 55% humidity). After a 2-wk acclimatization period, 18 male C57BL/6.KOR-ApoE<sup>shl</sup> mice were randomly allocated to two groups (n = 9) and fed on the normal CE-2 diet or a diet supplemented with 10% CCH for 12 wk. Body weight and food consumption were monitored at regular intervals. At the end of 12 wks’ experiment, the mice were sacrificed, blood was obtained from veins and tissues were collected for further analysis. All animal procedures were carried out in accordance with the Animal Experimentation Guidelines of the Japanese Association for Laboratory Animal Science and were approved by the Animal Use and Care Committee of Nippon Meat Packers, Inc.

Plasma and hepatic total cholesterol (TC) and triglycerides (TG) were analyzed by using cholesterol E and TG E kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Low-density lipoprotein cholesterol (LDL-c) and high-density lipoprotein cholesterol (HDL-c) levels were determined by HDL and LDL/VLDL cholesterol assay kits (BioVision Inc., California, Mountain View, USA). Total lipids were extracted from aliquots of liver and analyzed by the method of Folch et al. (7) and dissolved in isopropanol.

Plasma levels of interleukin-6 (IL-6) and soluble intercellular adhesion molecule-1 (sICAM-1) were measured with mouse sandwich enzyme-linked immunosorbent assay (ELISA) kits for cytokine (R&D Systems Inc., Minneapolis, MN, USA). Tumor necrosis factor-α (TNF-α) concentration was determined by using an
ELISA kit for cytokine (Shibayagi Co. Ltd., Gunma, Japan).

All data are presented as means±SE. The Fisher test was used and p values less than 0.05 were considered to be statistically significant.

Results and Discussion

During all of the experiments, monitoring results revealed that the amount of water drunk and food consumed as well as bodyweight gains were similar between the control group and CCH treatment group (data not shown), indicating that there was no significant trophicity for CCH administration.

The mice were treated with CCH for 12 wk and sacrificed for analysis. Compared with those of the control, the amount of plasma TC, hepatic TC and TG in the CCH group was reduced by 14.4, 24.7 and 42.8%, respectively (Table 1). However, CCH administration had no obvious influence on the concentration of TG, LDL-c, or HDL-c in plasma or TC in liver. C57BL/6.KOR-ApoE−/− mice are characterized by high plasma cholesterol levels, and high hepatic cholesterol and TG levels (8, 9). Our previous study indicated that CCH was absorbed with peptides in blood through enzymatic digestion (10). Present results suggested that CCH had a lipid-lowering effect through regulation of hepatic lipid biosynthesis to suppress TG levels in addition to lowering plasma TC. It is possible to surmise that the peptides of CCH were absorbed as a regulatory factor to influence cholesterol homeostasis. However, we cannot explain why LDL-c was not significantly changed directly by CCH administration. Further research is needed to elucidate these metabolic regulation mechanisms.

To investigate whether CCH had a preventive and therapeutic effect on atherosclerosis, atherosclerotic lesions in the aorta were observed by microscopy and the Oil Red O staining method (Fig. 1A). There were no obvious differences in the aortas between the CCH and control group. Then we tested whether CCH treatment can alleviate liver damage in the C57BL/6.KOR-ApoE−/− mouse model. Sections of paraffin-embedded liver were stained with hematoxylin-eosin or Oil Red O. Treatment with 10% CCH for 3 mo decreased the abundance of diffuse lipid droplets and fat vacuoles compared with those of the control group (Fig. 1B, C).

Because inflammation is now considered an important mechanism of atherosclerosis (11), markers of inflammation were also examined as a function of dietary interventions. In the present research, the effect of CCH treatment on plasma proinflammatory cytokine levels in C57BL/6.KOR-ApoE−/− mice was investigated by the methods of ELISA (Fig. 2). Administration of CCH resulted in a decrement of IL-6 (by 43.4%, p<0.05), sICAM-1 (by 17.9%, p<0.05), and TNF-α (by 24.1%, p<0.01) levels in the plasma. IL-6, sICAM-1, and TNF-α are the main proinflammatory cytokines secreted by adipocytes. During the inflammatory response to pathogens in adipocytes, TG concentration in plasma was increased (12). In addition, the liver is a very complicated organ that is involved in cholesterol, TG, and glucose metabolism in association with inflammatory cytokines (13). Our results suggested that treatment of CCH downregulates several proinflammatory cytokines concerned with lipid metabolism in C57BL/6.KOR-ApoE−/− mice.

A previous study revealed that CCH has a strong ACE-inhibitory activity (4). CCH treatment could increase serum NO levels transiently in normal rats (6). NO can inhibit the expression of VCAM-1 and the proinflammatory cytokines IL-6 and TNF-α in the vas-
cular endothelium (14). On the other hand, CCH was characterized as richness in hydroxyproline. The peptide contained hydroxyproline from CCH possesses ACE-inhibitory activity, while single hydroxyproline or essential amino acids do not have this characteristic (10). From these results, we hypothesized that CCH administration resulted in an increment of ACE-inhibitory activity in vivo, which upregulated the synthesis of NO and triggered lipid-lowering metabolism in the liver, with a subsequent decrease in proinflammatory cytokine levels.

Although the present research results cannot confirm directly that CCH reduces the buildup of atherosclerosis plaque, the data demonstrated that feeding CCH substantially reduced both the total lipid content in the liver and the production of proinflammatory cytokines such as IL-6, TNF-α, and sICAM-1 in a model of elevated susceptibility to atherosclerosis. Our results suggest that long-term CCH administration could provide an incorporated, beneficial adjunctive therapy for the prevention and management of atherosclerosis or cardiovascular disease.

REFERENCES