A Novel Method of Utilization of By-products of Poultry Industry;

Oral Administration of Type-II Collagen from Chicken Cartilage Suppresses Adjuvant Arthritis in Rats

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BACKGROUND

From the global viewpoints of increasing population and worsening environment, a great deal of attention has been focused on utilization and recycling of limited resources.

By-products of meat industry should be utilized much more than ever. Bone and skin of domestic animals have been used as sources of collagen and gelatin to prepare foods and chemical and medical products. Cartilage, however, has rarely been utilized. The cartilage predominantly contains type-II collagen (CII), whereas bone and skin do collagen types I and III.

Many studies have suggested that oral administration of CII prevented experimental model animals from developing rheumatoid arthritis-like autoimmune diseases [1-4]. Such a phenomenon is called oral immune tolerance, by which such immune response as antibody production is never brought forth, even if animals are immunized with an antigen and again with the same antigen. It has been believed that native CII (NCII) or whole molecule of CII could induce the oral immune tolerance, but that heated and denatured CII (DCII) or cleaved molecule of CII could hardly do so [1].

Our previous study, however, elucidated that oral as well as intranasal administration of DCII and its fragments could inhibit collagen-induced arthritis (CIA) in model mice [5].

OBJECTIVES

The present study was carried out to examine (1) the effects of oral administration of CII, particularly DCII, on prevention of adjuvant arthritis (AA) in model rats and (2) the feasibility of utilization of chicken sternal cartilage, a by-product of poultry industry, as a CII source. AA is an experimental model of not only RA but also other joint inflammation-related diseases.

MATERIALS AND METHODS

Collagen: CII was extracted from chicken sternal cartilage with the limited pepsin-digestion method, purified with the salt-precipitation method and finally solved in 2.5 mM citric acid. Type-I collagen (CI) was similarly extracted from bovine tendon. DCI and DCII were prepared by heating CI and CII for 30 min at 60°C, respectively.

Preparation of AA model rats: Seven-w-old Lewis male rats were purchased from Charles River Japan, Inc. After a 1-w quarantine period, an adjuvant mixture consisting of 0.1-mg *Mycobacterium butyricum* powder (Difco, Detroit, MI) and 0.3-ml mineral oil was injected intracutaneously at each rat's tail base to prepare the AA model rats. Similarly, the same volume of PBS was injected intracutaneously (referred to as a normal rat).

Oral administration of collagen: Each 50-mg/kg portion of NCII, DCII or DCI suspended in 0.5% tragacanth gum was orally given to the AA model rat everyday from 1 through 23 days after the immunization. Similarly, only the tragacanth gum (referred to as vehicle) was orally given to the rats (Fig. 1).

Body weight: Body weight of each rat was determined every 2-3 days during the experimental period (Fig. 1).

Degree of arthritis: Volume of 2 hind paws of each rat was measured by the water-displacement method during the experimental period (Fig. 1).

Erythrocyte sedimentation rate (ESR): ESR, a pathological index of degree of inflammation, was conventionally determined at the end of the experimental period (Fig. 1).

Histopathological analyses: At the end of the experimental period, the animals were sacrificed. Affected paws were removed postmortem (Fig. 1), and then conventionally fixed, decalcified, embedded, sectioned, stained with haematoxylin and eosin and microscopically analyzed. Degrees of cellular infiltration, pannus proliferation, cartilage degradation, bone resorption and joint swelling were numerically scored from 1 (no alteration) to 5 (severe alteration) according to the method of E. M. O'Byrne *et al* [6].

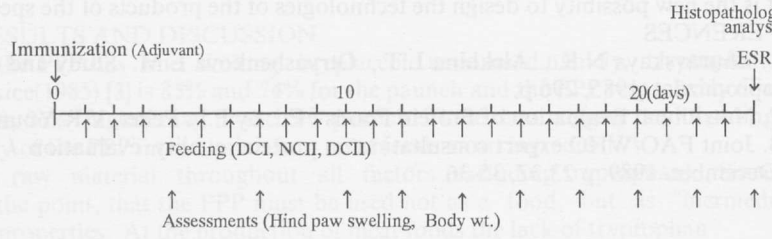

Fig.1 Experimental design

RESULTS

Prevention of AA by NCII and DCII: Oral administration of NCII and DCII significantly inhibited development of arthritis in the model rats, whereas that of DCI did not do so (Fig. 2). Likewise, body-weight gains of the rats were inhibited with the development of AA (data not shown).

ESR data suggested that oral administration of NCII and DCII significantly prevented systemic and/or peripheral inflammation, but that no oral administration of DCI did so (Fig. 3) . Furthermore, oral administration of DCII prevented the inflammation more significantly than that of NCII (p<0. 05).

Histopathological analyses of the affected paws: Such histopathological changes as cellular infiltration, pannus proliferation, cartilage degradation, bone resorption and joint swelling were numerically analyzed (Fig. 4). Sums of the histopathological scores of the DCI-administrated rats were not significantly different from those of the vehicle-administrated rats. However, the sums of the NCII- and DCII-administrated rats were significantly lower than those of the vehicle- and DCI-administrated rats. Furthermore, the sums of the histopathological scores of the DCII-administrated rats were significantly much lower than those of the NCII-administrated rats (p<0.001). Particularly, oral administration of DCII prevented the model rats from developing cellular infiltration.

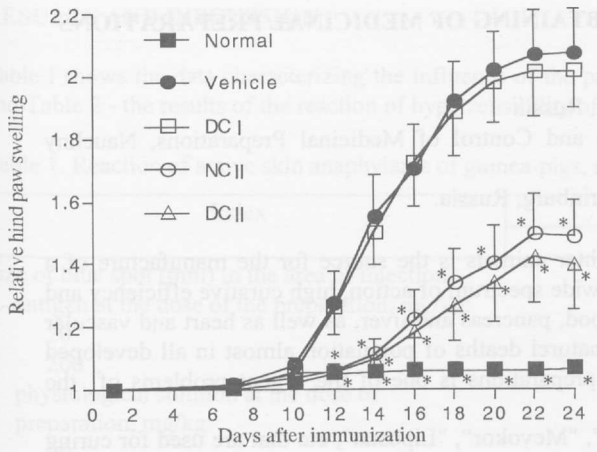

Fig.2 Effects of oral administration of DCI, NCII and DCII on hind paw swelling of the AA-model rats
(Mean ± SEM, ∗ :p<0.001) As for the denaturation condition, see the text.

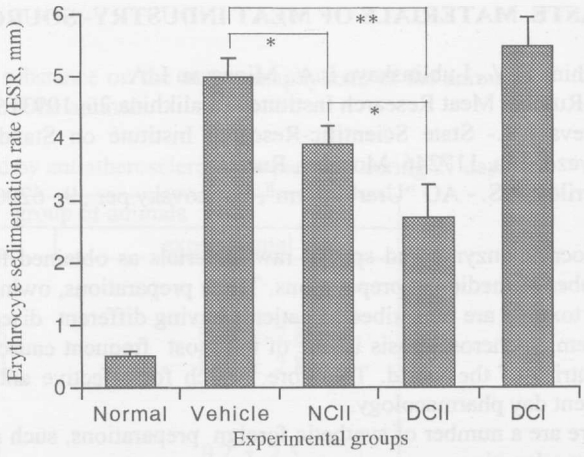

Fig.3 Effects of oral administration of DCI, NCII and DCII on erythrocyte sedimentation rate of the AA-model rats
(Mean ± SEM, ∗ :p<0.05, ∗∗ :p<0.01)

DISCUSSION

As for rheumatoid-arthritis (RA) therapy by oral administration of CII, clinical studies had been carried out in the U.S. and Europe. Significant therapeutic efficacy of CII was not always observed: one reason of such observation may depend on the fact that they had not used DCII but NCII. Anyhow, no side effect by oral administration of CII was reported [7-9].

We recently observed that (1) oral as well as intranasal administration of DCII inhibited CIA in mice, that (2) DCII inhibited production of IgG_{2a}, a complement cascade-reaction activator, and inflammation-causing cytokine of IFN-γ, but that (3) DCII promoted production of inflammation-suppressing cytokines of TGF-β and IL-10 [5].

The present study showed that oral administration of NCII and DCII inhibited AA in rats macroscopically, microscopically and pathologically, and that oral administration of DCII inhibited AA more significantly than that of NCII. The latter finding is of great advantage to the meat/poultry industry, for tolerogenicity of CII may be increased by pasteurization of CII.

CONCLUSIONS

From these results, (1) the feasibility of utilization of chicken sternal cartilage, a by-product of poultry industry, as a CII source was proved and (2) DCII is a promising candidate for medicine and/or functional foods to treat RA and other joint inflammation-related diseases.

Abbreviations;
CI, *type-I collagen*; **CII**, *type-II collagen*; **NCII**, *native type-II collagen*; **DCI**, *denatured type-I collagen*, **DCII**, *denatured type-II collagen*; **AA**, *adjuvant arthritis*; **CIA**, *collagen-induced arthritis*; **RA**, *rheumatoid arthritis*; **ESR**, *erythrocyte sedimentation rate*

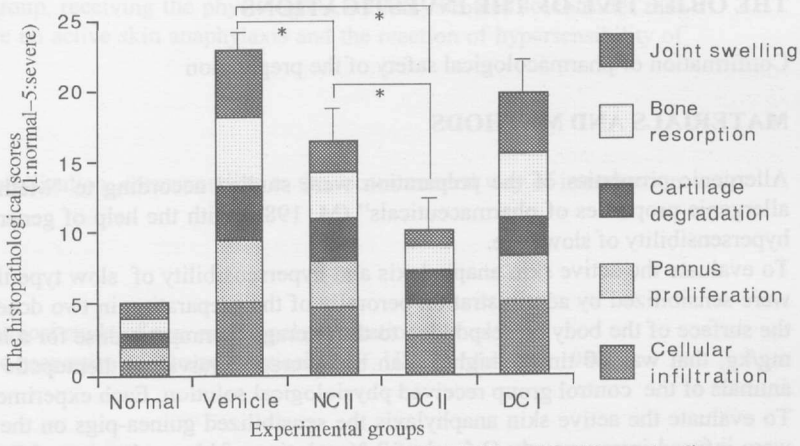

Fig.4 Effects of oral administration of DCI, NCII and DCII, on the five histopathological parameters of the AA-model rats (Mean ± SEM, ∗ :p<0.001)

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