8-P27

INTRANASAL ADMINISTRATION OF TYPE-II COLLAGEN SUPPRESSES COLLAGEN-INDUCED ARTHRITIS IN MICE AND ENHANCES THE EFFECT OF AN ANTI-INFLAMMATORY DRUG

Yasuki TAGUCHI[1], Takashi MATSUMOTO[1], Kotaro FUJITA[1], Fumiki MORIMATSU[1], Tamotsu SHIGEHISA[1], Shuichi KAMINOGAWA[2] and Ryoji YAMADA [1]

[1] Research and Development Center, Nippon Meat Packers, Inc., Tsukuba, Ibaraki 300-2646; and [2] Department of Applied Biological Chemistry, the University of Tokyo, Tokyo 113-8657, Japan

Background and Objectives:

Oral or intranasal administration of type-II collagen (CII) prevents laboratory animals from developing rheumatoid arthritis (RA), an autoimmune disease [1-3], by immune tolerance [4]. It has been considered that native CII (N.CII) and the whole molecule of CII may induce oral immune tolerance, but denatured CII (D.CII) or cleaved molecules of CII cannot do so [1]. Our previous study, however, showed that collagen-induced arthritis (CIA) in mice was prevented with D.CII and its fragments orally and intranasally administered before CIA induction [5]. The present paper describes (1) the therapeutic effect of CII intranasally administered after CIA induction and (2) synergistic suppression of CIA by the combined administration of CII and diclofenac, an anti-inflammatory drug.

Materials and Methods:

Collagen: CII was extracted from chicken sternal cartilage by limited pepsin-digestion and purified by the salt-precipitation method. Finally, CII was dissolved in 1.5 mM citric acid at a concentration of 10 mg/ml (referred to as "N.CII"). Autoclaved CII (referred to as "A.CII") was prepared by heating N.CII for 20 min at 121°C. Bovine CII, used for induction of CIA, was similarly extracted from bovine articular cartilage and dissolved in 10 mM acetic acid at a concentration of 4 mg/ml.

Anti-inflammatory drug: Diclofenac sodium (Sigma, St. Louis, MO), a nonsteroidal anti-inflammatory drug (NSAID) for RA treatment [6-7], was suspended in 0.5% tragacanth gum (Sigma) at a concentration of 0.6 mg/ml.

Animals: Female DBA/1J mice, 6 weeks old, were purchased from Charles River Japan (Yokohama, Japan).

Preparation of CIA model mice: Bovine CII was emulsified with CFA (Difco Laboratories, Detroit, MI) at a ratio of 1:1 (v/v). A 0.1-ml portion of the CII-CFA emulsion (200 μg CII) was intracutaneously injected at the tail base of each mouse (primary immunization). Then, a CII-IFA (Difco) emulsion was injected 21 days after the primary immunization.

***Intranasal administration of collagen*:** In line with our previous study [5], 200 μg of N.CII or A.CII was intranasally administered three times a week.

Oral administration of diclofenac: In line with our preliminary toxicity test (data not shown), diclofenac was orally administered three times a week (1 mg/kg body weight).

***Assessment of CIA*:** Arthritis was scored from 0 to 3 depending on the severity every 2-3 days during the experimental period. The sum of the four-paw scores of each mouse was defined as an arthritic index.

***Determination of anti-CII antibodies*:** On the 30th day from the primary immunization, serum samples were collected from the mice and anti-CII IgG1 and anti-CII IgG2a antibodies were determined by ELISA.

Results:

***Prevention of CIA with N.CII, A.CII and diclofenac*:** Intranasal administration of A.CII significantly suppressed CIA, but that of N.CII did not. Oral administration of diclofenac did not suppress CIA (Fig. 1). Such tendencies were coincidentally observed with regard to onset of CIA (data not shown).

***Synergistic prevention of CIA by combined administration of CII and diclofenac*:** Although individual administration of either N.CII or diclofenac did not suppress CIA, combined administration of the both significantly suppressed CIA. Similarly, the combined administration of A.CII and diclofenac prevented CIA more markedly than did individual administration of A.CII (Fig.1).

Anti-CII antibodies in affected mice: In three diclofenac-administered groups, production of anti-CII IgG1 antibody was low ($p<0.001$). No such a tendency was observed in the other groups except the A.CII-administered group ($p<0.05$). Reduced production of anti-CII IgG2a antibody was observed the in N.CII- and A.CII-administered groups, whereas no such a tendency was observed in the control nor the diclofenac-administered groups. Reduced production of anti-CII IgG2a was more marked in the A.CII group than N.CII group. Furthermore, combined administration of CII and diclofenac suppressed anti-CII IgG2a production more markedly than did individual administration of N.CII or A.CII (Fig. 2).

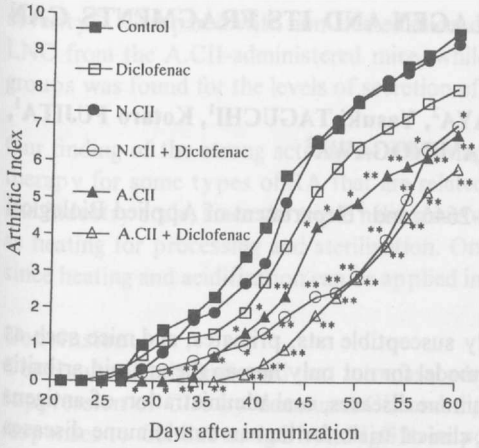

Fig.1 Effects of individual and combined administration of N.CII, A.CII and diclofenac on the severity of CIA in mice (* :$p<0.05$, ** :$p<0.01$ by the Mann-Whitney U test, compared with the control group)

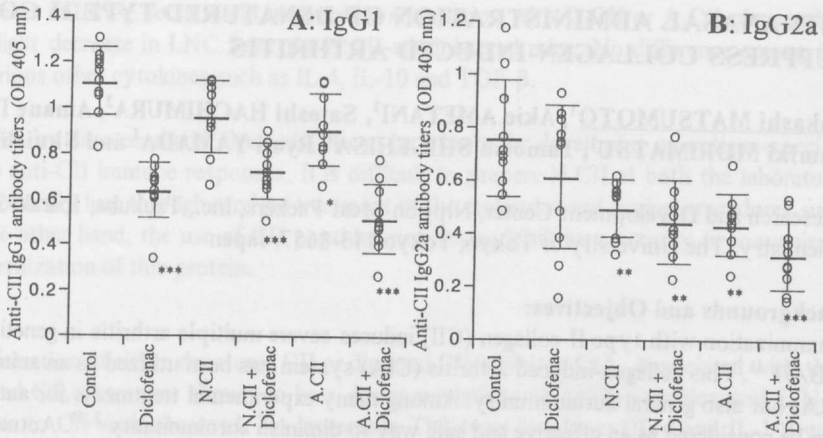

Fig.2 Effects of individual and combined administration of N.CII, A.CII and diclofenac on antibody production of anti-CII IgG1 (A) and anti-CII IgG2a (B) (Mean ± SD, * :$p<0.05$, ** :$p<0.01$ *** :$p<0.001$ by the Mann-Whitney U test, compared with the control group.)

Discussion:

We observed that intranasal administration of A.CII lessened severity and suppressed onset of CIA in mice and A.CII suppressed production of IgG2a antibody, a complement-cascade-reaction activator [5]. Such suppression occurred even after CIA induction, suggesting that intranasal administration of A.CII works not only preventively but also therapeutically. Moreover, the combined administration of N.CII and diclofenac and that of A.CII and diclofenac suppressed CIA more markedly than did the individual administration of N.CII, A.CII or diclofenac.

Conclusion:

(1) Intranasal administration of A.CII effectively suppressed CIA in mice even after CIA induction, suggesting a therapeutic effect of A.CII;
(2) Efficacy of A.CII was not inferior to that of diclorenac, an anti-inflammatory drug, and;
(3) Combined administration of CII and diclofenac synergistically suppressed CIA.

Pertinent literature:

1. Nagler-Anderson, C., Bober, L. A., Robinson, M. E., Siskind, G. W. and Thorbecke, G. J. *Proc. Natl. Acad. Sci.* **83**, 7443-7446, 1986.
2. Zhang, Z. J., Lee, C. S. Y., Lider, O. and Weiner, H. L. *J. Immunol.* **145**, 2489-2493, 1990.
3. Al-Sabbagh, A., Miller, A., Santos, L. M. B. and Weiner, H .L. *Eur. J. Immunol.* **24**, 2104-2109, 1994.
4. Weiner, H. L., *Immunol. Today* **18**, 335-343, 1997.
5. Matsumoto, T., Ametani, A., Hachimura, S., Iwaya, A., Taguchi, Y., Fujita, K., Shigehisa, T., Yamada, R. and Kaminogawa, S. *Proc. 45th ICoMST. pp - ,* 1999
6. Weisman, M. H. *Clin. Ther.* **8**(4), 427-438, 1986.
7. de Vries, B. J. and van den Berg, W. B. *J. Rheumatol. Suppl.* **18**, 10-18, 1989.