Enhancing Activity of Anthranilic Acid on Adjuvant Arthritis in Rats and Antibody Formation in Mice

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Accepted May 24, 1989

Abstract—Anthranilic acid (ANA), a metabolite of tryptophan, was examined for its immunopotentiating properties. Administration of ANA (12 mg/kg/day, p.o.) significantly enhanced the development of adjuvant arthritis in rats, although not in a dose-related manner. ANA tended to enhance adjuvant disease moderately suppressed by pretreatment with cyclophosphamide (CY), an immunosuppressive agent. ANA (3-30 mg/kg/day, p.o.) also caused a dose-related enhancement in the antibody formation to sheep erythrocytes (SRBC) in mice.

Anthranilic acid (ANA) is one of the metabolites of tryptophan and a classical vitamin (vitamin L1). There are few reports about the physiological activity of ANA, except for its action to prolong the life span of adrenalectomized rats (1). Its derivatives such as mfenamic acid and fulfenamic acid are used as nonsteroidal anti-inflammatory drugs. Recently, some compounds related to ANA have been developed for clinical use. One of them is tranilast (N-5'), an anti-allergic drug, that can inhibit antigen-induced release of chemical mediators from mast cells (2); and another is lobenzarit, an antirheumatic agent, that has immunomodulating activity (3). However, there has been no report about the effect of ANA itself on the inflammation reaction and/or immune response.

In the present study, we investigated the effect of ANA on adjuvant arthritis in rats, a model of immunologically induced chronic inflammation, and on the antibody production in mice immunized with sheep erythrocytes (SRBC).

Male Fischer rats (6 weeks old) and male BALB/c mice (10-11 weeks old) obtained from Japan SLC, Inc. (Hamamatsu) were used. They were housed in an air-conditioned room at a constant temperature and humidity environment and given standard rodent pellets (MF, Oriental Yeast) and water ad libitum. Adjuvant arthritis was induced by a single s.c. injection with 0.6 mg Mycobacterium butyricum (Difco Laboratories, Detroit, MI) suspended in 0.1 ml of liquid paraffin into the tail of the rats. Both hind paw volumes were measured with a plethysmometer (TK-101, UNICOM, Chiba) by water displacement just before adjuvant injection (day 0) and then at appropriate intervals in order to follow the development of the inflammatory edema. The percent increase in paw volume was calculated relative to the initial value on day 0. ANA (Nacalai Tesque, Inc.) was administered p.o. in doses of 4, 12 and 36 mg/kg/day starting from the day of adjuvant injection and then at appropriate intervals in order to follow the development of the inflammatory edema. The percent increase in paw volume was calculated relative to the initial value on day 0. ANA (Nacalai Tesque, Inc.) was administered p.o. in doses of 4, 12 and 36 mg/kg/day starting from the day of adjuvant injection. Cyclophosphamide (CY) (Nacalai Tesque, Inc.) was dissolved in saline immediately before use, and it was administered i.p. in a dose of 30 mg/kg 2 days before adjuvant injection. Antibody forming cells in the spleen were measured by the modified method of Cunningham's hemolytic plaque assay (4). Briefly, mice were immunized with i.p. injection of 5×10⁸ SRBC (Nippon Biosupp. Center, Tokyo). Four days later, the mice were killed by cervical dislocation, and their spleen cells were examined for the number of anti-SRBC plaque-forming cells (PFC). ANA was administered p.o. in doses of 3, 10 and 30 mg/kg/day starting from the day of immunization of SRBC. The results
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Effects of ANA on the development of adjuvant arthritis in rats: untreated (A) and CY-pretreated (B). Rats were injected s.c. with 0.6 mg adjuvant (Mycobacterium butyricum) into the base of the tail. Various doses of ANA were administered p.o. once a day from the day of adjuvant injection to the end of the experiment. CY (30 mg/kg) was given i.p. 2 days before adjuvant injection. Each point represents the mean±S.E. of 7–9 rats. *P<0.05, **P<0.01, as compared with the arthritic control.

As shown in Fig. 1A, the daily treatment with ANA enhanced the symptoms of adjuvant-induced arthritis after day 17. The enhancing effect was not dose-related and attained its maximum with p.o. administration at a dose of 12 mg/kg/day. Since adjuvant arthritis is thought to occur through a cell-mediated autoimmunity to the proteoglycan component of rat cartilage tissues (5), we further tested for the effect of ANA on adjuvant arthritis diminished by pretreatment with CY, an immunosuppressive agent (6). The results in Fig. 1B show that ANA tends to enhance the adjuvant disease moderately suppressed by the treatment with CY, although the difference was not statistically significant. Figure 2 shows the primary antibody formation to SRBC in mice receiving ANA for four consecutive days. ANA caused a dose-related enhancement in the number of direct (IgM) PFC in the spleen, and a significant difference was detected at doses of 10 and 30 mg/kg. ANA treatment had little influence on the food intake and growth rate of animals in both experiments.

The observations that ANA augments the adjuvant arthritic lesions and the antibody production to SRBC at doses that do not possess anti-inflammatory activities (data not shown) suggest that ANA exhibits immunopotentiating properties. The mechanism by which ANA enhanced the immune responses is presently unclear. Some reports have described that antirheumatic drugs with immunomodulating properties, such as levamisole and D-penicillamine, enhance the development of arthritis induced by adjuvant as well as by type II collagen in rats (7, 8), although levamisole causes a dose-related suppression of adjuvant arthritis augmented by thymectomy in rats (8). Also, such drugs have been reported to lead to a potentiation of delayed type hypersensitivity in mice (9).

Alteration of the tryptophan metabolism in patients with rheumatoid arthritis has been observed by Igari et al. (10). Furthermore, recent reports have shown that the degradation of tryptophan in human macrophages can be induced by interferon-γ and interleukin-2 (11, 12), and that it is correlated...
Effects of ANA on anti-SRBC PFC formation in mice. Mice were i.p. injected with $5 \times 10^8$ SRBC. ANA was administered p.o. once a day for 4 days after immunization as shown in the figure. The control group was given water p.o. Each column represents the mean±S.E. of 4 mice. *P<0.05, **P<0.01, as compared with the control.

closely to the release of neopterin, which is considered to be a marker for activated cell-mediated immunity.

Although ANA has been considered as one of the endproducts of tryptophan and is excreted in the urine after conjugation (13), it can partly change to 3- or 5-hydroxy derivatives (14), which are also metabolites of tryptophan. Thus, ANA or its metabolites might play some physiologically significant role in the immune system. Further studies are needed to determine whether the effects of ANA are due to a direct action or to a secondary influence on the immune process.

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