N-(2-Carboxyphenyl)-4-chloroanthranilic acid disodium salt: a novel anti-arthritic agent without anti-inflammatory and immunosuppressive activities

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Adjuvant arthritis in rats is well known as a chronic model of inflammation and has usually been employed in the evaluation of anti-arthritic drugs (Winter, 1965). Cell-mediated immunity is considered to be important for the development of adjuvant arthritis (Eugui & Houssay, 1975; Pearson & Wood, 1959, 1964; Waksman, Pearson & Sharp, 1960; Waksman & Wennersten, 1963). It has been reported that anti-inflammatory and immunosuppressive agents possess prophylactic or therapeutic activity in adjuvant arthritis (Winter, 1965; Glenn, 1966; Winter & Nuss, 1966; Currey & Ziff, 1968; Perper, Alvarez & others, 1971; Walz, DiMartino & Misher, 1971; Martel, Klicius & Herr, 1974). In this article we describe a new type of anti-arthritic agent, *N*-(2-carboxyphenyl)-4-chloroanthranilic acid disodium salt (CCA), which inhibits adjuvant arthritis in rats, in spite of its lack of both anti-inflammatory and immunosuppressive activities.

Male Sprague-Dawley rats (8 weeks old) were purchased from Clea Japan, Inc. Adjuvant arthritis was induced by intradermal injection of 0·5 mg of heat-killed *Mycobacterium tuberculosis* cells suspended in liquid paraffin into the tails of rats.

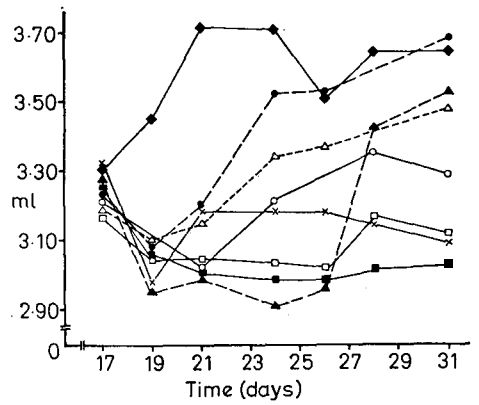

FIG. 1. Therapeutic effect of CCA on adjuvant arthritis in rats. Oral administration of CCA and phenylbutazone was started on day 17 and continued for 6 more days (a total of 7 doses). Control ◆—◆, CCA (mg kg^{-1}) 0·1, ●--●, 1 ○—○, 5 ×—×, 10 □—□, 50 ■—■, 100 △--△. Phenylbutazone 10 mg kg^{-1} ▲--▲. Ordinate—Hind paw volume (ml), values represent the mean of 10 rats per group. Abscissa—Time (days after adjuvant injection).

* Correspondence.

The prophylactic effect of CCA on induction of adjuvant arthritis is shown in Table 1. A daily dose of 100 mg kg^{-1} of CCA dissolved in water was administered orally for 25 consecutive days starting from 3 days before adjuvant injection to the end of the experiment. Control animals were given water. The rats were examined at intervals of 2 or 3 days after adjuvant inoculation to record the incidence and the severity of the arthritis. The lesions of the four paws were each graded from 0 to 4, as described by Koga & Pearson (1973). Volumes of hind paws were measured 19 and 21 days after adjuvant. CCA decreased the incidence and the severity of the arthritis, and hind paw volume compared with controls. No lesion was observed up to 12 days after adjuvant.

Fig. 1 shows the therapeutic effect of CCA. In this experiment, the drug was administered for 7 consecutive days from 17 to 23 days post-adjuvant injection. Just before initiation of treatment animals were distributed into groups of ten on the basis of hind paw volume and body weight so that the groups were as similar as possible. In the controls, paw volume increased up to 21 days thereafter staying constant. In contrast, CCA treated animals, given an oral dose of 0·1–100 mg kg^{-1} day^{-1}, had a significant decrease

Table 1. *Prophylactic effect of CCA on adjuvant arthritis induction in rats.*

Adjuvant arthritis	Days after adjuvant inoculation: 12	14	17	19	21
Control Incidence[a]	3/10	7/10	7/10	8/10	9/10
Severity[b] (mean score ± s.e.)	0·55 ± 0·23	1·30 ± 0·45	2·10 ± 0·66	3·15 ± 0·81	5·72 ● 1·33
Hind paw vol. (mean ± s.e.)	N.D.	N.D.	N.D.	3·03 ± 0·10	2·77 ± 0·15
CCA Incidence	3/10	3/10	3/10	4/10	3/10
Severity (mean score ± s.e.)	0·45 ± 0·24	0·75 ± 0·35	0·70 ± 0·26	1·35 ± 0·78	1·90 ± 0·87*
Hind paw vol. (mean ± s.e.)	N.D.	N.D.	N.D.	2·18 ± 0·11*	2·08 ± 0·09**

Adjuvant was injected at day 0. A daily dose of 100 mg kg^{-1} of CCA was administered orally from 3 days before adjuvant injection to the end of the experiment.

[a] Number of rats with arthritis out of ten.

[b] The maximal score is 16.

N.D. = Not determinated.

Statistically significant difference from controls, $*P < 0.05$ $**P < 0.01$.

of paw volume in a few days. The optimal dose is 10 to 50 mg kg^{-1}, within this range the volumes of hind paws diminished most and remained low after treatment stopped. At higher or lower dosages, the potency of the drug decreased, the paw volume increasing after treatment with 0·1, 1 or 100 mg kg^{-1} day^{-1}. Phenylbutazone, as a standard drug was effective at an oral dose of 10 mg kg^{-1} day^{-1}, while, unlike CCA, paw volumes returned to control values after the treatment.

It was well known that most anti-arthritic drugs hitherto reported, except for a few drugs such as penicillamine, possess an anti-inflammatory and/or immunosuppressive activity. However, CCA has no anti-inflammatory activity either in the carrageenan-induced paw oedema assay or in the cotton pellet granuloma test. CCA has no effect on tuberculin skin reaction and passive cutaneous anaphylaxis in rats and guinea-pigs, respectively. Furthermore, CCA has no specific inhibitory effect on phagocytic function or chemotaxis of macrophages and neutrophils *in vitro*.

Although the precise mechanisms of the development of adjuvant arthritis still remain unknown, an immunological process is thought to be involved, possibly a cell-mediate immune response. Treatment with heterologous anti-lymphocyte serum (Currey & Ziff, 1968) or immunosuppressive agents such as cyclophosphamide and 6-mercaptopurine (Glenn, 1966) is known to prevent the induction of this disease. However, CCA has immunoenhancing, as opposed to immunosuppressive activity. An increase in haemolytic plaque forming cells of mouse spleen immunized with sheep red blood cells, DNP-Ficoll, or lipopolysaccharides, and enhancement of blastoid transformation of mouse spleen cells and thymus cells by concanavalin A were brought about by the drug both *in vitro* and *in vivo*.

Also, as shown in Table 2, CCA treatment of rats with adjuvant arthritis from 17 to 21 days post-adjuvant injection resulted in the prevention of atrophy

Table 2. *Effect of oral administration of CCA on thymus weight in adjuvant arthritis rats.*

Group and dose (mg kg^{-1})	No. of rats	Thymus wet weight (mg)/ body weight (g) (Day 22)	Hind paw volume (ml)	
			Before treatment (Day 17)	After treatment (Day 22)
Control —	10	0·864 ± 0·104	3·17	3·79 ± 0·24
CCA 10	10	0·944 ± 0·084	3·05	3·14 ± 0·11*
CCA 50	10	1·211 ± 0·100**	3·07	2·92 ± 0·11*
Normal —	5	1·210 ± 0·170		

CCA was administered orally for 5 consecutive days from 17 to 21 days post-adjuvant injection. Values indicate the mean ± s.e.
Statistically significant difference from controls, $*P < 0·01$, $**P < 0·05$.

of the thymus which can be generally observed in such rats. As the mean weight of thymus in the CCA-treated group was comparable with that of intact animals, it is suggested that CCA might prevent atrophy of the thymus. That CCA acted directly on the gland and prevented it from atrophy or stimulated the proliferation of thymus cells, may be considered. Since phenylbutazone also restored thymic weights, the possibility that improvement of the lesions after drug treatment may reduce the stress and indirectly prevent thymic atrophy, cannot be ruled out.

Recently Kayashima, Koga & Onoue (1976) reported that thymectomy and/or low dose (200 R) irradiation at the young adult age led to a striking enhancement in the induction of rat adjuvant arthritis. They also described the importance of the thymus-derived cells in the regulation of induction of the arthritis. The cells are short-lived, radiosensitive and resemble suppressor T lymphocytes.

From these points of view, one possible mechanism of CCA's inhibitory effect on adjuvant arthritis may be related to stimulation of thymus, and the consequent enhancement of the regulatory function of the thymus-derived lymphocytes on adjuvant arthritis induction.

April 9, 1977

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