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A modification of rat adjuvant arthritis for testing antirheumatic drugs

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An inflammation having an acute and a prolonged phase was induced by the injection of carrageenan into the paw of rats inoculated with adjuvant 6 days previously (adjuvant-carrageenan-induced inflammation, ACII). Steroidal and acidic non-steroidal anti-inflammatory drugs were effective on both phases. Basic non-steroidal anti-inflammatory drugs were effective only on the acute phase, and cytotoxic drugs only on the prolonged phase. Neither gold nor chloroquine preparations inhibited either phase. Effectiveness of drugs on the acute phase was similar to that on carrageenan oedema. Inhibitory effects of drugs on the prolonged phase were similar to those on the secondary lesions of adjuvant arthritis. The ACII test is considered to have advantages compared with the adjuvant arthritis test in duration of drug administration, in the measurement of intensity of inflammation, in the time for experiment and in analysing the action of drugs.

The evaluation of drug activity on adjuvant arthritis is relatively complicated and the data are not always reliable. Mizushima, Tsukada & Akimoto (1970) induced an inflammation of the rat paw having an acute and a prolonged phase by injection of carrageenan into the paw of rats inoculated with adjuvant three or more days before the carrageenan (adjuvant-carrageenan-induced inflammation, ACII). It was considered that the prolonged phase of ACII was produced through a mechanism common to that of the secondary lesions of adjuvant arthritis. We have now examined the effects of several antirheumatic and anti-inflammatory drugs on ACII to find if this model is suitable for testing such compounds.

MATERIALS AND METHODS

Animals

Random-bred female rats of the Sprague-Dawley strain aged from 8 to 11 weeks and weighing 200 to 280 g were used.

Induction of adjuvant-carrageenan-induced inflammation (ACII)

Liquid paraffin (0·1 ml) (Merck, Germany) containing 0·6 mg of heat-killed *Mycobacterium butyricum* (Difco) was injected intradermally into the basal part of the tail. A suspension in saline of 0·1 ml of carrageenan (1–1·5%) (Nitto kaiso, Tokyo) was injected in the subplantar region of the left hindpaw of the rats six days after the adjuvant inoculation. A concentration of carrageenan was chosen such that 3 or 4 h after its injection there was an increase in paw volume of at least 80%. Any type of carrageenan that produced an adequate swelling and lymphoid cell infiltration appeared to be suitable.

The volume of the left hindpaw was measured before and after the carrageenan injection according to Van Arman, Begany & others (1965). The percentage inhibi-

tion of the acute (3 h after carrageenan injection) and prolonged phase (72 h after the injection) of ACII produced by the drugs was calculated from the increase in paw volume as follows.

Inhibition on acute phase (%)

$$= \frac{\text{Control (mean)} - \text{treated rat}}{\text{Control (mean)}} \times 100$$

Inhibition on prolonged phase (%)

$$= \frac{\text{Control (mean)} - \text{treated rat}}{\text{Control (mean)} - \text{control without adjuvant inoculation}} \times 100$$

Drugs

The drugs used were: dexamethasone, hydrocortisone acetate and indomethacin (Merck); prednisolone, sodium thiomalate and cyclophosphamide (Shionogi, Osaka), phenylbutazone (Fujisawa, Osaka); flufenamic acid (Kowa, Nagoya); aspirin and aminopyrine (commercial), benzydamine HCl (Dai-ichi, Tokyo); tinoridine hydrochloride (Yoshitomi, Tokyo); chloroquine diphosphate (Bayer); methotrexate (Lederle); mercaptopurine (Takeda, Osaka); azathioprine (Wellcome). Methotrexate, cyclophosphamide and gold thiomalate were dissolved in saline and azathioprine and mercaptopurine were dissolved in NaOH and then diluted with saline. The other drugs were suspended or dissolved in 0·5% carboxymethylcellulose suspension except for benzydamine HCl which was dissolved in water. A dose of the drug solutions of 0·5 ml/100 g was administered to the rats. Control animals were given the same volume of saline or suspension.

Methods

The animals were fasted from the evening of day 5 to just after the carrageenan injection (day 6). All drugs, except gold and chloroquine, were administered either 1 h before (day 6), or 1 h before and 24 h after (day 6, 7), or 24 h and 1 h before and 24 h after (day 5, 6, 7) carrageenan injection as indicated in Table 1.

RESULTS

Course of ACII

The course of ACII and carrageenan oedema is shown in Fig. 1. The intensity of swelling of the paw in ACII was similar to that of carrageenan oedema about 3 h after the injection. However, the inflammation was prolonged and the swelling increased again 1 to 3 days after the injection. We have called the early inflammation the acute phase and the later prolonged inflammation the prolonged phase. The prolonged phase of ACII was induced by the carrageenan injection in 80 or 90% of animals in most experiments. When carrageenan was not injected into the rats inoculated with adjuvant, the usual secondary lesions of adjuvant arthritis developed in approximately 90% of the animals.

Effects of antirheumatic or anti-inflammatory drugs on ACII

The effects of some steroidal and non-steroidal anti-inflammatory drugs, cytotoxic drugs, gold and chloroquine preparations on the acute and prolonged phases of ACII

are summarized in Table 1. The doses of drugs chosen were similar to or a little higher than those used in usual animal experiments. Steroidal and non-steroidal anti-inflammatory drugs produced approximately 50% inhibition of the acute phase at the dose chosen. All of the steroidal and non-steroidal anti-inflammatory drugs strongly suppressed the acute phase. Steroidal and acidic non-steroidal anti-

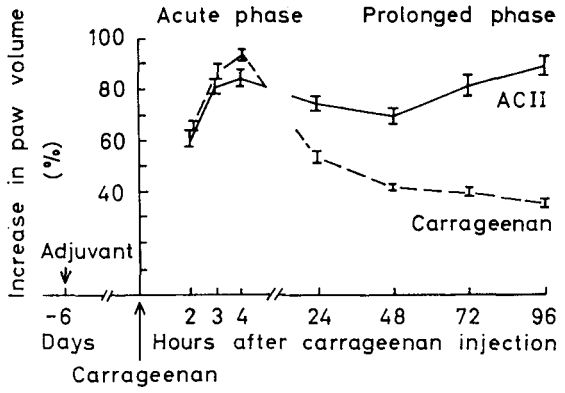

FIG. 1. Course of ACII and carrageenan oedema in rats. ACII (adjuvant-carrageenan-induced inflammation) was induced by the injection of carrageenan on 6th experimental day in the paw of rats inoculated with adjuvant on 0 day. Carrageenan oedema was induced in normal rats. The data were average values of 14 experiments (135 rats) ± standard errors.

Table 1. *Inhibitory effects of antirheumatic and anti-inflammatory drugs on acute and prolonged phase of ACII (adjuvant-carrageenan-induced inflammation) in rats.*

	Dose (mg/kg)	Route	Day drugs given	No. of rats	Inhibition (% ± s.e.) Acute phase	Prolonged phase
Steroidal						
Dexamethasone	0·1 × 2	Oral	6, 7	10	56 ± 3·3*	98 ± 5·5*
	0·1	,,	6	10	54 ± 2·8*	60 ± 8·9*
Prednisolone	5 × 2	,,	6, 7	10	56 ± 3·4*	110 ± 18·9*
Hydrocortisone	30 × 2	,,	6, 7	10	46 ± 5·0*	63 ± 11·7*
Acidic non-steroidal						
Indomethacin	2 × 2	,,	6, 7	10	59 ± 5·8*	100 ± 8·8*
	4 × 2	,,	6, 7	9	61 ± 5·9*	115 ± 4·3*
	4	,,	6	10	58 ± 2·4*	80 ± 9·1*
Phenylbutazone	30 × 2	,,	6, 7	10	38 ± 5·4*	81 ± 14·7*
	50 × 2	,,	6, 7	10	58 ± 5·9*	82 ± 11·7*
	50	,,	6	10	46 ± 4·7*	83 ± 7·3*
Flufenamic acid	10 × 2	,,	6, 7	10	44 ± 4·9*	82 ± 8·5*
	20	,,	6	10	52 ± 5·6*	73 ± 10·0*
Aspirin	150 × 2	,,	6, 7	10	53 ± 4·2*	90 ± 13·8*
	150	,,	6	10	53 ± 3·6*	37 ± 18·0
Basic non-steroidal						
Benzydamine HCl	100 × 2	,,	6, 7	9	50 ± 4·5*	25 ± 15·6
	100	,,	6	10	62 ± 7·7*	17 ± 22·9
Aminopyrine	100 × 2	,,	6, 7	9	64 ± 4·1*	19 ± 18·1
	100	,,	6	10	65 ± 2·3*	27 ± 19·8
Tinoridine HCl	100 × 2	,,	6, 7	10	59 ± 4·2*	−38 ± 18·8
Sodium thiomalate	40 × 8	i.m.	0–7	10	12 ± 5·4	−67 ± 42·4
	5 × 8	,,	0–7	10	19 ± 6·4	13 ± 16·8
Chloroquine diphosphate	50 × 8	Oral	0–7	10	17 ± 7·6	23 ± 29·2
Cytotoxic drugs						
Methotrexate	0·3 × 3	i.p.	5, 6, 7	9	7 ± 2·0	95 ± 5·9*
	0·3 × 2	,,	5, 6	10	8 ± 4·7	83 ± 10·1*
Cyclophosphamide	10 × 3	,,	5, 6, 7	8	8 ± 4·1	101 ± 4·4*
	10 × 2	,,	5, 6	10	11 ± 4·6	58 ± 10·0*
Mercaptopurine	20 × 3	,,	5, 6, 7	17	19 ± 3·4*	59 ± 13·4*
Azathioprine	30 × 3	i.m.	5, 6, 7	17	10 ± 2·4	73 ± 11·5*

ACII was induced by the injection of carrageenan on 6th experimental day in the paw of rats inoculated with adjuvant on day 0.

Inhibitory effects on acute phase and prolonged phase were measured at 3 (day 6) and 72 h (day 9) after carrageenan injection respectively.

* Significance ($P < 0.05$) in relation to control group.

inflammatory drugs also strongly inhibited the prolonged phase especially if administered on two days. On the other hand basic anti-inflammatory drugs, which showed rather stronger inhibitory effects on the acute phase than acidic drugs, gave no significant inhibitory effects on the prolonged phase. Gold thiomalate and chloroquine diphosphate suppressed neither the acute nor the prolonged phase, even though they were given for 8 days. Cytotoxic drugs markedly suppressed the prolonged phase, but did not inhibit the acute phase except for the weak inhibitory effect of mercaptopurine.

DISCUSSION

Steroidal anti-inflammatory drugs and acidic non-steroidal drugs such as indomethacin, flufenamic acid and phenylbutazone have both prophylactic and therapeutic effects on adjuvant arthritis (Winter & Nuss, 1966; Ward & Cloud, 1966; Graeme, Fabry & Sigg, 1966; Glenn, 1966). The three basic anti-inflammatory drugs in Table 1 did not suppress significantly the development of adjuvant arthritis at a daily dose of 100 mg/kg for 14 days (Akimoto, Tsukada & Mizushima, unpublished observation). Some cytotoxic drugs, which prevent the development of the secondary lesions (Ward, Cloud & others, 1964; Graeme & others, 1966; Glenn, 1966) and have beneficial effects on human rheumatoid arthritis, did not suppress established adjuvant arthritis (Whitehouse, 1969; Whittington, 1970). Chloroquine (Ward & Cloud, 1966; Graeme & others, 1966) and gold salt (Jessop & Currey, 1968) appear to exert no demonstrable activity at a therapeutic dose. Therefore, the inhibitory effects of drugs on the prolonged phase of ACII (Table 1) were similar to their prophylactic effects on adjuvant arthritis. We measured the inhibitory effects of drugs on the prolonged phase of ACII 72 h after the carrageenan injection (day 9). The effect may be measured on day 8 or 10. However the inhibitory effect of drugs on the swelling itself seemed to remain on day 8 and standard errors appeared to increase on day 10.

The acute phase of ACII is probably similar to carrageenan oedema. Inhibitory potencies of anti-inflammatory and antirheumatic drugs on the acute phase were similar to those obtained in carrageenan oedema.

Though adjuvant arthritis is established as an experimental model for testing antirheumatic drugs, the experiment takes two weeks or more and is complicated for drug evaluation. Drugs must usually be given for a week or more. In some cases a long term treatment causes a toxic reaction in animals which makes drug evaluation difficult. Chloroquine was active only in a nearly toxic dose (Winter & Nuss, 1966). The lethal dose of most cytotoxic drugs was close to the therapeutic dose in a long term treatment (Ward & others, 1964). In the ACII test, one or two doses of drug and a few simple measurements of paw volume were found to be sufficient to demonstrate drug activities and the experiments were complete within 10 days. In the ACII test the prolonged phase followed the acute phase which was considered to be a simple acute inflammation. The property of this model may be helpful for the analysis of drug action. For example the fact that cyclophosphamide did not influence the acute phase of ACII suggested that the marked inhibition of the prolonged phase by cyclophosphamide was due to neither a non-specific anti-inflammatory effect nor a toxic effect of the drug. On the other hand, basic non-steroidal anti-inflammatory drugs appeared only effective on simple acute inflammation.

These results suggest that the ACII test is useful for testing anti-inflammatory drugs despite the fact that both adjuvant and carrageenan must be injected and that two groups of controls, ACII and carrageenan oedema, are necessary for drug evaluation.

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