Suppression of adjuvant-induced arthritis in the rat by gold sodium thiomalate

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Gold salts have been used in the treatment of rheumatoid arthritis for nearly 40 years. Their effectiveness, while not consistent, has been well established (Freyberg, 1966) and their use continues. Recently, conflicting results concerning the effectiveness of gold salts on adjuvant arthritis in rats have been reported (Newbould, 1963; Jessop and Currey, 1968). Adjuvant arthritis, a crippling deformity resulting from diffuse connective tissue involvement, is considered by some authorities to be the best available experimental model of rheumatoid arthritis because of its strong clinical and pathological similarities to the human disease (Pearson, 1963), and because it can be effectively treated with most antiarthritic drugs it is widely used to evaluate potential therapeutic agents. By employing highly inbred (Lewis) rats as our experimental animals and utilizing a volume-displacement apparatus to measure the arthritic lesions objectively, we have obtained an arthritic model that is highly sensitive in detecting anti-arthritic activity.

In light of conflicting reports in the literature, we have re-evaluated the effectiveness of gold salts in adjuvant arthritis in rats under our test conditions.

Material and methods

ADJUVANT ARTHRITIS

Adjuvant was prepared by homogenizing desiccated *Mycobacterium butyricum* (Difco Laboratories, Detroit, Mich.) in white paraffin oil (NF) to give a suspension of 7.5 mg. *M. butyricum*/ml. Adjuvant arthritis was produced by a single intradermal injection of 0.1 ml. of adjuvant, on Day 0, into a footpad of the left hindpaw of male Lewis inbred rats (Microbiological Associates, Maryland) weighing between 138 and 195 g. The rats were anaesthetized with ether inhalation before and during the adjuvant injection.

The injected hindlegs became inflamed (increased leg volume) and reached their greatest size within 3 to 5 days (primary lesion). Adjuvant arthritis (secondary lesions) occurred after a delay of approximately 10 days and was characterized by inflammation of the non-injected sites

(right hindleg, forepaws, ears, nose, and tail) and further increases in the volume of the injected hindleg.

HINDLEG VOLUME

This was determined by a modified method of Van Arman, Begany, Miller, and Pless (1965). On the day of assay (3 to 4 hours after drug administration), the hindlegs were immersed to the anatomical hairline into a mercury reservoir. The mercury column was connected to a Statham pressure transducer (Model P23BB, 0.5 cm. Hg.). The output from the transducer was led through an amplifier to a Hewlett-Packard digital voltmeter (Model HP-3440A), and a high gain/auto range unit (Model 3443A), and finally to a digital recorder (Model J74562A). The digital recordings were calibrated, and a linear relationship between MV and ML was obtained by placing cylinders of known volumes into the mercury reservoir.

ARTHRITIC SCORE

The severity of secondary lesions in the uninjected hindleg was graded 0 to 4, depending upon the area and magnitude of involvement.

DRUG ADMINISTRATION

Gold sodium thiomalate (Myocrysine®) was prepared at various concentrations in 0.5 per cent. benzyl alcohol and injected intramuscularly (right thigh) in a volume of 0.5 ml./kg. The drug was administered once daily (exclusive of weekend days indicated in the text) starting either on Day 0 or one week before adjuvant injection and continuing until the termination of the experiment (Day 16). Control animals were treated in an identical manner using 0.5 per cent. benzyl alcohol for the intramuscular injections. The benzyl alcohol solution was prepared with either 0.9 per cent. saline or water for injection.

The level of significant difference between hindleg volumes, arthritic score, and body weight of drug-treated and control groups was determined by the Student's 't' test.

SERUM GOLD LEVEL

Blood samples were obtained after the decapitation of the adjuvant arthritic rats. 0.5 ml. aliquot of serum was diluted to 3.0 ml. with distilled water and absorption measured in a Perkin-Elmer Model 303 atomic absorption spectrophotometer (wavelength 243.3 mμ slit 4,

lamp source 20 m.a., acetylene-air mixture and single-slot burner head) using a recorder read-out attachment and chart recorder. Peak height on the chart was converted to percentage absorption and gold levels calculated by linear regression using standard curves of aqueous aurothioglucose.

Results

INITIAL EXPERIMENTS

The data in Table I show the effects produced by 5 and 10 mg./kg. gold sodium thiomalate on the primary and secondary lesions of adjuvant arthritis in three independent experiments. Gold sodium thiomalate administered at a dose of 10 mg./kg. produced a slight but inconsistent suppression of the primary lesion (injected leg—Day 3). The effects of gold sodium thiomalate on the secondary lesions, however, were more consistent. On Day 16, the injected leg volumes were significantly decreased at the 10 and 5 mg./kg. doses; whereas the 10 mg./kg. dose also significantly suppressed the development of secondary lesion in the uninjected leg. Pre-treating the rats with gold sodium thiomalate one week before adjuvant injection did not appear markedly to alter the results, with the exception of producing significant suppression of the injected leg volume by Day 10.

DOSE RANGE STUDY

The effects of gold sodium thiomalate at doses of 40, 20, 10, and 5 mg./kg. on the primary lesion, secondary lesions, and serum gold levels in adjuvant arthritic rats are illustrated by graphs in Figs 1, 2, and 3. Suppression of the primary lesion produced by gold sodium thiomalate, although slight, appears to be dose-related (Fig. 1).

FIG. 1 Effect of various doses of gold sodium thiomalate on the primary lesion (injected hindleg volume—Day 3) of adjuvant arthritis. Mean of six rats per dose level. Vehicle control hindleg volume (mean $\pm$ S.E.) 3.02 ± 0.07 ml.

Fig. 2 shows the dose-related suppression of the secondary lesions produced by gold sodium thiomalate. All doses of gold sodium thiomalate produced statistically significant suppression of the injected hindleg volume on Day 16, whereas only the 20 mg./kg. dose produced a significant effect on the uninjected hindleg volume in this experiment. The relationship between daily doses of gold sodium thiomalate and gold levels in serum (collected on Day 17) is illustrated in Fig. 3. There was a significant negative correlation ($P \leq 0.05$) between serum gold levels and the severity of the secondary lesion in the uninjected leg volume on Day 16.

FIG. 2 Effect of various doses of gold sodium thiomalate on the secondary lesions (hindleg volumes—Day 16) of adjuvant arthritis. Mean of (3), (4), or (6) rats per dose level.

Vehicle control hindleg volume (mean $\pm$ S.E.) 4.30 ± 0.11 ml. (injected leg) and 2.57 ± 0.23 ml. (uninjected leg).

FIG. 3 Serum gold levels after administration of various doses of gold sodium thiomalate to adjuvant arthritic rats (serum obtained on Day 17). Mean and range of (3), (4), or (6) rats per dose level.

Discussion

The results of these experiments show that gold sodium thiomalate injected into the rat inhibits both the primary and secondary lesion of induced adjuvant arthritis. It could be argued that the decreases in leg volumes are a reflection of decreases in body weight. That this is not the case is shown in Tables I and II. In these studies body weights of drug-treated rats were not significantly less than body weights of vehicle-treated rats. Pretreatment of the rat with gold sodium thiomalate one week before adjuvant injection produces essentially the same effects as those seen when gold sodium thiomalate therapy was initiated on the same day as the induction of adjuvant arthritis. These results are in agreement with the observations of Newbould (1963) on the effectiveness of gold sodium thiomalate in adjuvant-induced arthritis and also strengthen and extend his observations by showing that there is a dose-response relationship in suppressing adjuvant arthritis and elevating serum gold levels. These results are not, however, in agreement with reported

Table II Comparison of body weights in dose range study

Drug	Dose (mg./kg./day)	Body weight (g.) (mean ± S.E.)	
		Day 3	Day 16
Gold sodium thiomalate	40	157±6	197*±8
	20	162±5	196*±4
	10	163±3	177 ±4
	5	168±6	179 ±6
Control vehicle	—	167±2	177 ±3

*P ≤ 0.05 Significant increase from control vehicle.

observations of Jessop and Currey (1968), who could not produce an effect on adjuvant-induced arthritis in rats pretreated with gold sodium thiomalate. Since the doses of gold sodium thiomalate used in our laboratory were similar to most of those used by

Table I Effect of gold sodium thiomalate on primary and secondary lesions (hindleg volumes) in adjuvant arthritis

Days† drug administered	Drug	Dose (mg./kg./day)	No. of rats per group	Arthritic score (uninjected leg)	Body weight (g.)	Hindleg volume (ml.)			
						Injected leg			Uninjected leg
						Day 3†	Day 10	Day 16	Day 16
-7 to +16	Gold sodium thiomalate	10	6	2.00±0.58	179±11	3.19±0.04	2.65±0.15	3.76±0.36	2.50±0.28
	Control vehicle	—	8	3.12±0.48	168±6	3.31±0.18	3.42±0.18	4.48±0.16	3.42±0.29
0 to +16	Gold sodium thiomalate	10	6	1.50±0.22	178±5	3.14±0.10	2.80±0.10	3.62±0.12	2.42±0.11
		5	6	2.67±0.42	161±3	3.23±0.09	2.90±0.09	3.93±0.11	2.86±0.07
	Control vehicle	—	6	2.83±0.31	166±4	3.28±0.06	3.14±0.20	4.60±0.12	3.17±0.23
		—	8	2.75±0.31	160±4	2.87±0.07	3.29±0.34	3.97±0.19	2.80±0.28
0 to +16	Gold sodium thiomalate	10	6	1.83±0.31	167±5	2.49±0.11	2.84±0.04	3.16±0.19	2.14±0.15
		5	6	2.33±0.21	173±3	2.88±0.06	2.87±0.18	3.34±0.10	2.39±0.06
	Control vehicle	—	6	2.83±0.31	166±4	3.28±0.06	3.14±0.20	4.60±0.12	3.17±0.23
		—	8	2.75±0.31	160±4	2.87±0.07	3.29±0.34	3.97±0.19	2.80±0.28

All values represent mean and standard error of six to eight rats.
† Days after adjuvant injection—exclusive of days -3, -2, +4, +5, +11, +12.
* P ≤ 0.05 Significant decrease from control vehicle.
** P ≤ 0.01

Jessop and Currey (1968), the divergent results may be due to a difference in experimental protocol. Some of the differences between our study and Jessop and Currey's work were strain of rat, adjuvant material, dosing regimen, and methods of evaluating the arthritic syndrome. It is known that both severity and response to therapy of the adjuvant-induced arthritis are affected by the strain of rat used (Swingle, Jaques, and Kvam, 1969; Pelczarska, 1969).

The fact that adjuvant-induced arthritis in rats is suppressed by steroidal and non-steroidal anti-arthritic agents (Winter and Nuss, 1966; Graeme, Fabry, and Sigg, 1966), immunosuppressive drugs

(Graeme, Fabry, and Sigg, 1966), and chrysotherapy tends to confirm the utility of this disease model.

Summary

Parenteral gold, which has been shown to be effective in treating rheumatoid arthritis, was found to be effective in suppressing adjuvant-induced arthritis in the rat. Gold sodium thiomalate in a dose-related manner significantly suppressed both the primary and secondary lesions and raised the serum gold levels. The effectiveness of chrysotherapy in this experiment further supports the belief that the adjuvant-induced arthritic rat is a useful model for testing antiarthritic agents.

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