SODIUM AUROTHIOMALATE, GOLD KERATINATE, AND VARIOUS TETRACYCLINES IN MYCOPLASMA-INDUCED ARTHRITIS OF RODENTS

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IN the late 1930s mycoplasma-induced arthritis was described in laboratory rats (Findlay *et al.*, 1939) and mice (Sabin, 1939). The disease in rats was shown to be caused by a mycoplasma which at the time was termed L4 but which is now known as *Mycoplasma arthritidis*. Sabin (1939) described at least three different mycoplasma species that would cause arthritis in mice and designated them types A, B and C. Types A and C have now been re-named *Mycoplasma neurolyticum* (type A) and *Mycoplasma pulmonis* (type C). Type B, which was reputed to cause a chronic proliferative arthritis in mice resembling, particularly in its pathological characteristics, the human rheumatoid joint, was lost and has never been recovered. These observations, together with the finding (Findlay, Mackenzie and MacCallum, 1940; Sabin and Warren, 1940) that mycoplasmal arthritis in mice responded to gold therapy, led to speculation that mycoplasmas might be involved in the pathogenesis of rheumatoid arthritis and prompted a number of workers to investigate models of mycoplasma-induced arthritis in rodents (Preston, 1942; Tripi and Kuzell, 1947; Parkes and Wrigley, 1951). In later years, these same observations induced some investigators to try to isolate mycoplasmas from the joints of rheumatoid patients (Stewart *et al.*, 1974).

Early attempts to produce arthritis in rodents were fraught with difficulty, as the mycoplasmas were found frequently to lose virulence and usually had to be administered with adjuvants such as agar or as tumour material, containing *M. arthritidis*, obtained from rats (Woglom and Warren, 1938; Jasmin, 1957*a*). Nevertheless, between 1940 and 1960 numerous chemotherapeutic and medicinal agents were evaluated in these models (Powell and Rice, 1944; Powell, Jamieson and Rice, 1946; Kuzell and Gardner, 1950; Jasmin, 1957*b*), and it was re-affirmed that gold salts were effective in preventing mycoplasma-induced arthritis. In addition, it was found that chlortetracycline had a marked prophylactic effect on mycoplasma-induced arthritis in rats (Brown *et al.*, 1949). Because of this, tetracyclines were tested in rheumatoid arthritis and were claimed to have beneficial effects (Brown, Bush and Felts, 1959; Sanchez, 1968). However, this was not confirmed in controlled clinical trials with tetracycline HCl (Skinner *et al.*, 1971), and the results of this study, contrasting with the sensitivity of mycoplasmas to tetracyclines *in vitro*, have led many to conclude that mycoplasmas are not concerned in the aetiology of rheumatoid arthritis.

In recent years, a number of new reproducible models of mycoplasma-induced arthritis have been described (Schütze, 1968; Barden and Tully, 1969; Cole *et al.*, 1971; Hannan and Hughes, 1971) which have enabled drugs to be evaluated in both acute and chronic forms of mycoplasmal arthritis. This

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paper reports the re-investigation of sodium aurothiomalate and gold keratinate, together with various tetracyclines, in two of these animal models.

MATERIALS AND METHODS

Mycoplasmas. *M. arthritis* no. ATCC14124 was obtained from the American Type Culture Collection, Rockville, Md, USA. *M. pulmonis* strain JB was obtained from Dr J. G. Tully, National Institutes of Health, Bethesda, Md, USA, as a glucose-broth culture. The organisms were grown in mycoplasma broth (see below) for 48 h at 37°C and then stored, in 1-ml amounts, at -70°C; on thawing, these cultures contained c. 10^5 colony-forming units (c.f.u.) per ml. For in-vivo experiments, one vial was used to seed 100 ml of mycoplasma broth, which was then incubated for 48 h before being injected into animals. Viable counts were carried out by the method of Miles, Misra and Irwin (1938). Challenge doses of *M. arthritis* and *M. pulmonis* ranged from 9×10^7 to 4.5×10^8 c.f.u. and 1.2×10^3 to 3.3×10^7 c.f.u., respectively.

Mycoplasma medium. Liquid medium for the growth of mycoplasmas consisted of Heart Infusion Broth (Difco) 70% (v/v) horse serum no. 3 (Wellcome Reagents Ltd) 20% (v/v), and 25% extract of fresh yeast (Distillers Co. Ltd) 10% (v/v). Penicillin G was added to the medium to give a final concentration of 200 units per ml. When solid medium was required, Bacto Agar (Difco) was added to the Heart Infusion Broth to a concentration of 1.4% (w/v) before sterilisation. Broth and agar were sterilised by autoclaving at 15 lb per sq. in. for 15 min.

Animal models. The models of mycoplasma-induced arthritis used in these experiments were described previously: for rats infected with *M. arthritis* by Hannan and Hughes (1971), and for mice infected with *M. pulmonis* by Barden and Tully (1969). The disease in rats is an acute polyarthritis which reaches maximal severity 7 to 8 days after infection, while that in mice is more chronic and may persist, in some mice, for periods of over a year.

Choice of animals. Preliminary experiments revealed that the incidence, severity and persistence of arthritis were greatly influenced by the strain of animal used. Even animals of one strain obtained from different breeders showed marked differences in their susceptibility to arthritis after the same mycoplasmal challenge. As a result of these investigations female, specific-pathogen-free (SPF) Sprague-Dawley rats weighing 150-170 g (Charles Rivers, Seine-Maritime, France) and female, cesarean-derived, outbred albino NIH mice (Oxfordshire Laboratory Animal Colonies, Bicester, Oxfordshire) weighing 16-20 g were selected for subsequent experiments. Mycoplasmas were not detected in the lungs or nasopharynx of these animals before use. The ID₅₀ (median infective dose) of *M. arthritis* in the Sprague-Dawley rats was c. 1×10^7 c.f.u. (Hannan and Hughes, 1971), while in NIH mice doses of *M. pulmonis* ranging from 1×10^2 to 1×10^7 c.f.u. regularly caused severe persistent arthritis in every infected animal. Intravenous injection of sterile mycoplasma broth into these strains of rats and mice did not cause arthritis.

Induction and assessment of arthritis. Arthritis was induced in rats by injecting 0.5 ml per 100 g of body weight of a 48-h broth culture of *M. arthritis* into the tail vein. Mice were infected similarly with *M. pulmonis*, except that 0.5 ml of a 1 in 100 dilution of a 48-h broth culture was injected. Groups of eight animals were used throughout. In all experiments, the number of arthritic joints (radiocarpal, tibiotarsal, metacarpophalangeal, metatarsophalangeal and interphalangeal) in each group was recorded at various times after infection. The mean number of arthritic joints was determined by dividing the total number of swollen joints by the number of animals in the group. In mouse experiments for the investigation of therapeutic activity, mice were selected, 21 days after infection, to provide groups with equivalent degrees of arthritis. The percentage reduction in arthritis after drug treatment was calculated by the following formula:

$$\frac{\text{mean number of arthritic joints on day 21} - \text{mean number of arthritic joints after treatment}}{\text{mean number of arthritic joints on day 21}} \times 100.$$

The degree of inflammation (erythema and oedema) was also assessed.

Preparation and administration of drugs. Sodium aurothiomalate (ATM) (May and Baker Ltd, Dagenham, Essex), gold keratinate (Johann A. Wülfing, Neuse, W. Germany) and five different tetracyclines, tetracycline HCl, oxytetracycline HCl, methacycline HCl (Pfizer, Sandwich, Kent), chlortetracycline HCl and demethylchlortetracycline HCl (Lederle, Gosport, Hants), were investigated *in vitro* and also for prophylactic and therapeutic activity in each model of mycoplasmal arthritis. The gold-containing drugs were administered as single subcutaneous injections, 1 day before infection, 3 days after infection, or 21 days after infection. Tetracyclines were given orally or subcutaneously, starting 1 h before infection, at the time of infection, or 21 days after infection; treatment was then continued daily for a further 6 days.

Drugs for oral administration were suspended in sterile 0.7% methyl cellulose solution and injected directly into the stomach through a stainless-steel cannula (Anderman and Co. Ltd, East Molesey, Surrey) inserted into the oesophagus. For other routes of administration, gold-containing drugs were dissolved in sterile, de-ionised water, and the tetracyclines were either dissolved or suspended in phosphate-buffered saline, pH 7.4.

Minimal inhibitory concentration. The MIC of the drugs was determined in Microtitre plates (Cooke Engineering Co., Flow Labs., Irvine, Scotland), by a modification of the metabolic-inhibition test (Taylor-Robinson, 1967). Drugs were serially diluted in sterile

TABLE I

Minimum concentration of sodium aurothiomolate (ATM), gold keratinate and various tetracyclines required to inhibit growth of Mycoplasma arthritis and M. pulmonis for periods of 2, 3 and 7 days

Drug	Period of inhibition (days)	Minimum inhibitory concentration (μg per ml) for	
		<i>M. arthritis</i>	<i>M. pulmonis</i>
ATM	2	7.8	15.6
	3	500.0	31.25
	7	>4000.0	62.5
Gold keratinate	2	62.5	500.0
	3	4000.0	1000.0
	7	4000.0	2000.0
Tetracycline HCl	2	0.03	<0.001
	3	0.06	<0.001
	7	1.0	0.004
Demethyl chlortetracycline HCl	2	0.004	<0.001
	3	0.015	<0.001
	7	0.06	0.002
Oxytetracycline HCl	2	0.125	0.004
	3	1.0	0.015
	7	3.9	1.0
Methacycline HCl	2	0.004	<0.001
	3	0.015	<0.001
	7	0.125	0.125
Chlortetracycline HCl	2	0.015	<0.001
	3	0.5	0.007
	7	0.5	1.0

de-ionised water to give a range of concentrations, either 125–0·001 μg per ml or 4000–15·6 μg per ml. Mycoplasma broth containing either 1% (w/v) of arginine monohydrochloride or 1% (w/v) of glucose, and 0·005% (w/v) of phenol red, was added at a strength to compensate for its dilution by the aqueous drug solution. Approximately 10^3 c.f.u. of *M. arthritidis* or *M. pulmonis* were added to each concentration of drug. Drug-free infected, non-infected and pH control wells were included on each plate. Plates were sealed with Sellotape and incubated at 37°C. MICs were recorded after 2, 3 and 7 days' incubation. The MIC was read as the lowest concentration of drug that prevented the colour change in the medium caused by the metabolism of the respective substrate by the mycoplasmas.

*Mycoplasma*static and *mycoplasma*cidal determinations. Comparisons of the mycoplasmastatic and mycoplasmaacid activity of the drugs were made by subculturing the contents of Microtitre wells showing no pH change in the MIC tests into 10-ml volumes of mycoplasma broth, and incubating them at 37°C; in the case of wells known to contain high concentrations of drug a further dilution with 10 ml of mycoplasma broth was made 2 days later, to remove any remaining mycoplasmastatic drug activity. If no colour change occurred in these subcultures after 8 days' incubation, they were further subcultured on to mycoplasma agar, incubated at 37°C in an atmosphere of 95% N₂ and 5% CO₂ for 14 days, and then examined microscopically for mycoplasma growth. In this way the lowest concentration of a drug from which no mycoplasma could be re-isolated was determined; this constituted the minimal mycoplasmaacid concentration (MCC).

Gold assays. Gold levels in the sera of rats and mice were measured with an Eel 240 atomic absorption spectrophotometer fitted with a gold-sensitive lamp.

Tetracycline assays. Blood levels of tetracycline HCl, methacycline HCl and chlortetracycline HCl were determined in arthritic mice 21 days after infection with *M. pulmonis*. Each drug was given as a single dose, either 200 mg per kg of body weight subcutaneously, or 300 or 600 mg per kg orally, to groups of four or five mice. Blood samples were collected from the axillary region 10, 20, 30, 60, 120, 240 and 360 min. and 24 h afterwards, into heparin. They were tested for antibacterial activity by the cup-plate technique, with *Bacillus cereus* as the test organism; standard solutions for the tests were prepared in heparinised mouse blood. Plates were incubated aerobically at 30°C. The tetracycline content of the samples was derived from the regression line for the standard solutions.

Statistics. Differences in the reduction of arthritis between drug-treated mice and untreated arthritic mice were evaluated statistically by the one-tailed Mann-Whitney "U" test (Siegel, 1956).

RESULTS

*MIC tests and mycoplasma*static and *mycoplasma*cidal activity

The MICs of ATM, gold keratinate and the five tetracyclines for *M. arthritidis* and *M. pulmonis* are shown in table I. ATM and gold keratinate were found to have only slight antimycoplasma activity in these tests. The MIC of ATM for *M. arthritidis* after 2 days' incubation was 7·8 μg per ml but the inhibitory effect disappeared on further incubation. Gold keratinate was virtually inactive against *M. pulmonis*. The "zoning" phenomenon, which occurs in tests for antimycoplasma activity of gold-containing compounds against certain mycoplasma species (Newnham and Chu, 1965; Davidson and Thomas, 1966), was not observed with *M. arthritidis* or *M. pulmonis*. Some difficulty was experienced, however, in obtaining consistent end-points in MIC titrations with the gold-containing drugs. Similar difficulties in assessing the in-vitro activity of ATM against human urogenital-tract mycoplasmas and against numerous strains of *M. gallisepticum* have been reported previously (Robinson, Wichelhausen and Brown, 1952; Newnham and Chu, 1965). In

TABLE II

Minimum inhibitory concentration (MIC)* and minimum mycoplasmacidal concentration (MCC)* of ATM and three tetracyclines for *M. arthritis* and *M. pulmonis* after 2 days' and 14 days' exposure

Organism	ATM			Tetracycline HCl		
	MIC at 2 days	MIC at 14 days	MCC at 14 days	MIC at 2 days	MIC at 14 days	MCC at 14 days
<i>M. arthritis</i>	100†	100	10 000	1†	10	1000
<i>M. pulmonis</i>	100†	100	10 000	0.1†	1	10

Organism	Methacycline HCl			Chlortetracycline HCl		
	MIC at 2 days	MIC at 14 days	MCC at 14 days	MIC at 2 days	MCC at 14 days	MCC at 14 days
<i>M. arthritis</i>	1†	10	1000	1†	100	1000
<i>M. pulmonis</i>	0.1†	10	100	1†	10	1000

* μg per ml.

† The differences between the 2-day MIC values in this experiment and those in table I are caused by the dilution technique. The earlier results were obtained by diluting the drugs in Microtitre plates with Microtitre dilutors; this has an inherent "carry-over" effect, leading to lower MIC values. In the present experiment, more accurate dilutions were made in test tubes, with fresh pipettes for each dilution stage.

contrast to the gold-containing compounds, the tetracyclines displayed activity at very low levels against *M. arthritis* and *M. pulmonis* even after 7 days' incubation. It can be seen from table II that all the drugs examined were predominantly mycoplasmastatic in action. Although the MICs were low for all the tetracyclines after 2 days' incubation, these drugs only depressed mycoplasmal growth, and in most cases mycoplasmas could be re-isolated from very high concentrations of all of the drugs tested. In every case there was a 10–100-fold increase in the MIC between days 2 and 14, in comparison with the MCC. The MCC of 10 mg per ml for ATM against both *M. arthritis* and *M. pulmonis* was very high indeed.

Effect of gold compounds on mycoplasma-infected rats and mice

In rats infected with *M. arthritis* and mice infected with *M. pulmonis*, ATM afforded protection against the development of arthritis. In rats a single dose of 60 mg of ATM per kg of body weight, administered intramuscularly 1 day before infection, completely inhibited the development of arthritis, while 20 mg per kg was partially effective (fig. 1a). Similar results were recorded in *M. pulmonis*-infected mice, 20 mg per kg affording total protection and 6 mg

FIG. 1a and b.—The response of rats infected with 4.5×10^8 c.f.u. of *Mycoplasma arthritis* to a single dose of ATM given 1 day before (a) or 3 days after (b) infection. Groups of eight rats were given 6, 20 or 60 mg per kg of body weight, subcutaneously; $\square$ — $\square$ = 6 mg per kg; $\circ$ — $\circ$ = 20 mg per kg; $\triangle$ — $\triangle$ = 60 mg per kg; $\blacktriangledown$ — $\blacktriangledown$ = untreated rats.

FIG. 2.—Serum-gold levels related to the development of arthritis in rats infected with *M. arthritis*. Groups of eight rats, infected with 9×10^7 c.f.u., were given a single dose of 20 or 60 mg of ATM per kg of body weight, subcutaneously, one day before infection; $\square$ — $\square$ and $\square$... $\square$ = mean blood levels and mean number of arthritic joints after 20 mg per kg; $\circ$ — $\circ$ and $\circ$... $\circ$ = mean blood levels and mean number of arthritic joints after 60 mg per kg; $\blacktriangle$... $\blacktriangle$ = untreated rats.

per kg giving partial protection. In rats, treated 3 days after infection with *M. arthritidis*, the development of arthritis was checked by a dose of 60 mg per kg given intramuscularly and the arthritis was of lesser severity (fig. 1*b*). No therapeutic effect was observed in rats treated with 20 mg of ATM per kg.

Serum-gold determinations on rats treated with ATM showed that peak concentrations were reached 3.5–4 h after intramuscular injection. The mean serum-gold level at this time, after a dose of 60 mg per kg, was 95 μ g per ml, and after 20 mg per kg it was 46 μ g per ml. By 24 h these levels had dropped to 47.5 μ g per ml and 25.5 μ g per ml, respectively, and subsequently they gradually decreased further until, 1 wk after treatment, gold was undetectable

FIG. 3.—Response of mice with arthritis caused by *M. pulmonis* to a single dose of ATM or gold keratinate. Groups of eight mice, infected with 3.3×10^7 c.f.u., were given 180 mg of ATM or gold keratinate per kg of body weight, subcutaneously, 21 days after infection; ■—■ = ATM-treated mice; ●—● = gold-keratinate-treated mice; ▲—▲ = untreated mice.

in the serum. Fig. 2 shows the mean serum levels of gold over a 7-day period and relates them to the development of arthritis in rats infected with *M. arthritidis*. Although the serum-gold concentrations 1 day after infection were well above the MIC of this drug for *M. arthritidis* at 2-days' exposure *in vitro* only those rats treated with 60 mg per kg were protected completely. Arthritis developed in all rats treated with 20 mg per kg, but to a lesser extent than in the untreated, infected control rats.

Mice with severe arthritis that were treated 21 days after infection with *M. pulmonis* also responded to ATM therapy. After single doses of 60–240 mg per kg given intramuscularly, intravenously or subcutaneously, there was marked reduction in the number of swollen joints and in the degree of inflammation. This occurred gradually and was maximal 10–12 days after injection. Gold keratanate and ATM injected as single doses of 180 mg per kg subcutaneously

FIG. 4.—The effect of parenteral treatment with five different tetracyclines on the development of arthritis in rats infected with *M. arthritidis*. Groups of eight rats, infected with 3.3×10^8 c.f.u. were treated for 7 days with a daily dose of 100 mg of tetracycline per kg of body weight, subcutaneously, starting on the day of infection; ○- -○ = chlortetracycline HCl; ▽...▽ = demethyl chlortetracycline HCl; □- -□ = methacycline HCl; △- -△ = oxytetracycline HCl; ●—● = tetracycline HCl; ■...■ = untreated rats.

into groups of arthritic mice 21 days after infection demonstrated equal therapeutic activity, despite the inactivity shown by gold keratinate for *M. pulmonis* in in-vitro tests ($MIC > 500-2000 \mu g$ per ml). Both these drugs caused a considerable reduction in the number of arthritic joints (fig. 3) and in the degree of inflammation in infected mice; this effect was statistically significant for both drugs 18 days after treatment ($P < 0.05$). Serum samples collected for up to 5 h from mice given a single subcutaneous dose of 180 mg of gold keratinate per kg of body weight showed no antimycoplasmal activity against *M. pulmonis* in vitro, although gold concentrations of 26–44 μg per ml were detectable in the sera. These concentrations are equivalent to 200–338 μg of gold keratinate per ml, since gold constitutes *c.* 13% of the gold keratinate molecule.

Effect of tetracyclines

The results of treating *M. arthritidis*-infected rats at the time of infection with subcutaneous or oral doses of five different tetracyclines are shown in

FIG. 5.—The effect of oral treatment with five different tetracyclines on the development of arthritis in rats infected with *M. arthritidis*. Groups of eight rats, infected with 1×10^8 c.f.u., were treated for 7 days with a daily dose of 100 mg of tetracycline per kg of body weight, given orally and starting on the day of infection; ○--○ = chlortetracycline HCl; ▽...▽ = demethyl chlortetracycline HCl; □--□ = methacycline HCl; △--△ = oxytetracycline HCl; ●--● = tetracycline HCl; ■...■ = untreated rats.

TABLE III

The response of arthritic mice to subcutaneous tetracycline therapy starting 21 days after injection with M. pulmonis

Tetracycline (200 mg per kg of body weight)	Mean number of arthritic joints on the following days after infection:				
	21	34	37	41	44
Methacycline HCl	3.5	1.1*	1.5*	1.0*	1.0*
Chlortetracycline HCl	3.7	1.6	1.8*	1.1	0.6*
Demethyl chlortetracycline HCl	3.6	1.4	1.4†	1.4*	0.8†
Tetracycline HCl	3.2	1.0*	1.1†	0.6†	0.3‡
Oxytetracycline HCl	3.6	1.6	1.0†	1.0*	0.5†
No treatment	3.6	1.9	2.5	2.1	2.0

* $P = 0.05$; † $P = 0.01$; ‡ $P = 0.001$.

figs. 4 and 5. When given subcutaneously, each tetracycline, administered as 100 mg per kg of body weight daily for 7 days, completely prevented the development of arthritis. This prophylactic effect was maintained for a further 5 days after termination of treatment, but then some rats, in all groups except those treated with methacycline HCl, developed signs of arthritis. This, however, was less severe than in untreated, infected control rats. Identical experiments carried out in *M. pulmonis*-infected mice gave similar results, except that both methacycline HCl and chlortetracycline HCl protected mice for the entire duration of the experiment.

M. arthritis-infected rats treated orally with the tetracyclines, at a daily dose of 100 mg per kg, responded differently from those treated subcutaneously. Arthritis developed in all of them, and only in the group treated with chlortetracycline HCl was there a significant reduction in arthritis (fig. 5).

In *M. pulmonis*-infected mice treated with tetracyclines for 7 days, starting 21 days after infection, only subcutaneous injections, of 200 mg per kg daily, proved effective in reducing the mean number of arthritic joints and the degree of inflammation (table III and fig. 6). Mice treated orally, even with doses as high as 300 mg per kg daily, showed no change in the course of their arthritis, in comparison with untreated mice.

The mean blood levels of tetracyclines in arthritic mice, at various time intervals after subcutaneous or oral doses, are shown in table IV. Both tetracycline HCl and methacycline HCl were well absorbed, and the mean blood levels of these two drugs were considerably higher than in the case of chlortetracycline HCl. With tetracycline HCl, 200 mg per kg subcutaneously gave higher blood levels than 300 mg per kg orally. With methacycline HCl, similar blood levels were achieved after 200 mg per kg subcutaneously and 600 mg per kg orally. Low levels of chlortetracycline HCl were recorded in the blood of arthritic mice for both routes of administration throughout the course of the experiment.

DISCUSSION

Findlay *et al.* (1940) demonstrated that aurothioglucose prevented the development of *M. arthritidis* (L7)-induced arthritis in mice. The discovery that this gold compound was also effective *in vitro* against L7 preceded the finding by Preston, Block and Freyberg (1942) that it was the gold moiety of ATM that was responsible for its *in-vivo* anti-arthritic effect in mycoplasma-induced arthritis.

Since these early studies little has been published on the *in-vitro* antimycoplasmal activities of gold compounds other than ATM, and it is still generally

FIG. 6.—Response of mice with arthritis caused by *M. pulmonis* to a 7-days' course of treatment with chlortetracycline HCl or tetracycline HCl. Groups of eight mice with established arthritis were given seven daily doses of either 200 mg per kg of body weight subcutaneously (s.c.), or 300 mg per kg orally (p.o.), starting on the 21st day after infection; ●—● = 200 mg of chlortetracycline HCl s.c.; ■—■ = 300 mg of chlortetracycline HCl p.o.; ○—○ = 200 mg of tetracycline HCl s.c.; □—□ = 300 mg of tetracycline HCl p.o.; ▲—▲ = untreated mice.

TABLE IV
Blood levels of tetracycline HCl, methacycline HCl and chlortetracycline HCl in mice with M.pulmonis-induced arthritis after a single subcutaneous or oral dose

Drug	Dose (mg per kg)	Route of administration	Mean blood level ($\mu\text{g per ml}$)† after															
			10 min.		20 min.		30 min.		60 min.		2 h		4 h		6 h		24 h	
Tetracycline HCl	200	s.c.	31.5*	± 4.87	29.8	± 4.03	21.0	± 1.48	21.0	± 1.48	13.0	± 0.82	8.0	± 0.68	6.0	± 0.35	2.6	± 0.29
	300	p.o.	3.8	± 1.31	5.6	± 1.38	6.4*	± 1.20	5.3	± 1.21	3.3	± 0.98	1.9	± 0.58	1.8	± 0.70	<0.1	...
	600	p.o.	10.4*	± 3.68	7.9	± 0.79	7.3	± 0.73	10.2	± 0.71	7.3	± 0.64	6.9	± 1.04	7.4	± 1.48	<0.1	...
Methacycline HCl	200	s.c.	11.8	± 1.04	14.2*	± 1.83	11.2	± 2.13	12.7	± 1.59	13.6	± 2.86	8.1	± 0.61	6.9	± 0.59	1.2	± 0.08
	300	p.o.	7.9*	± 1.72	2.6	± 0.54	5.4	± 1.89	4.9	± 0.71	5.7	± 0.82	4.6	± 1.38	2.3	± 0.38	<0.1	...
	600	p.o.	13.5*	± 3.15	10.5	± 0.34	9.6	± 2.75	8.6	± 1.27	10.4	± 1.40	9.6	± 1.65	5.9	± 0.82	<0.1	...
Chlortetracycline HCl	200	s.c.	3.1	± 0.16	3.4*	± 0.32	3.2	± 0.36	3.0	± 0.29	3.1	± 0.35	3.0	± 0.30	3.3	± 0.55	0.81	± 0.23
	300	p.o.	1.7	± 0.36	1.6	± 0.21	1.7	± 0.27	2.1	± 0.22	2.9*	± 0.51	1.0	± 0.23	1.2	± 0.17	0.48	± 0.08
	600	p.o.	2.2	± 1.08	3.1	± 0.42	4.4	± 0.66	4.8*	± 1.14	3.4	± 0.18	3.1	± 0.48	2.1	± 0.36	<0.10	...

s.o. = Subcutaneously; p.o. = orally.

* Peak blood levels.

† Based on groups of five mice.

accepted that the antiarthritic effect of these drugs is due to an antimycoplasmal action. The results recorded in this paper, like those of Sabin and Warren (1940), do not support this contention.

In the experiments described here, only slight mycoplasmastatic activity was demonstrated *in vitro* for ATM against *M. arthritidis* and *M. pulmonis*. Gold keratinate was virtually inactive *in vitro* against *M. pulmonis*. In spite of this, both gold salts were effective in preventing or modifying arthritis induced by these mycoplasmas in rats and mice. Similarly, although ATM given prophylactically prevented the induction of arthritis in mice by Sabin's type-B mycoplasma, this organism was insensitive to ATM *in vitro* (Sabin and Warren, 1940). Others have also reported that ATM is relatively inactive against certain mycoplasmas (Stewart, Burnet and Young, 1969; Ford, 1972).

The discrepancy between the in-vitro antimycoplasmal activity of ATM and gold keratinate and their effect on mycoplasmal arthritis might be explained by metabolic conversion of these drugs into more active antimycoplasmal derivatives within the mouse. In these studies this did not seem to be the case. While the serum gold levels in rats treated with ATM were adequate to inhibit *M. arthritidis in vitro*, serum of mice under treatment with gold keratinate failed to suppress the growth of *M. pulmonis*, despite the presence of moderately high concentrations of gold. It is possible that an antimycoplasmal metabolite of gold keratinate was produced in the mice in very small amounts, and that this accumulated in the joints to cause an antimycoplasmal effect. Concentration of gold in joints has been described (Lawrence, 1961), and may occur in mice. However, at present, there is no evidence to support this idea, and the mode of action of gold keratinate in mycoplasmal arthritis is difficult to explain. The fact remains that gold compounds with poor to almost non-existent in-vitro antimycoplasmal activity are very potent antiarthritic agents.

In all, the results of these studies indicate that not all members of the Mycoplasmataceae are as sensitive to gold salts as was originally supposed and that gold-containing drugs vary in their antimycoplasmal activity. They also suggest that these drugs act, in mycoplasmal arthritis, not directly on the mycoplasmas but rather on the host's immunity mechanisms or on the inflammatory response.

Many other biological properties of gold have been described, including the stabilisation of lysosomal membranes (Weissman, 1966), the suppression of polymorphonuclear and macrophage phagocytosis and of macrophage migration (Vernon-Roberts, Jessop and Doré, 1973; Jessop, Vernon-Roberts and Harris, 1973), immunosuppression (Lorber *et al.*, 1974), C1 inactivation (Schultz *et al.*, 1974), and immunoenhancement (Measel, 1975). These properties alone, or in combination, might be responsible for the antiarthritic effect of gold salts in mycoplasmal arthritis. Undoubtedly, the effect would be enhanced if the gold-containing drug also possessed good antimycoplasmal activity.

In contrast to ATM and gold keratinate, all the tetracyclines tested displayed high levels of mycoplasmastatic activity against *M. arthritidis* and *M. pulmonis*. With each drug the mycoplasmacidal concentration was 10–100 times greater

than the mycoplasmastatic concentration. Nevertheless, all the tetracyclines were effective in preventing or delaying the onset of arthritis induced by these mycoplasmas in rats and mice, when administered subcutaneously. However, when given orally only chlortetracycline HCl and methacycline HCl were effective. The efficacy of chlortetracycline HCl is difficult to understand, since mouse-blood concentrations of this drug were considerably lower than those of the other tetracyclines, and its MIC against both mycoplasmas tended to be higher. These results illustrate that good antimycoplasmal activity and adequate drug absorption are not the only requirements for a drug to be effective in mycoplasmal arthritis. Other factors, such as drug transportation, availability, protein binding, and cell penetration, as well as good mycoplasmacidal activity, must play important roles in the efficacy of drugs in these diseases.

The route of administration of tetracyclines is obviously an important factor in the treatment of mice with established arthritis. Only mice treated by the subcutaneous route responded; daily oral therapy, even with doses as high as 300 mg per kg, failed to alter the course of the arthritis. These observations are of particular interest, because it was by the latter route that tetracycline HCl was administered to rheumatoid patients in a recent double-blind controlled study; a daily dose of 250 mg for one year had no beneficial effect on the course of arthritis in these patients (Skinner *et al.*, 1971). These results have been interpreted to mean that mycoplasmas are not causative agents in rheumatoid arthritis. The results obtained in our arthritic mice show clearly that if mycoplasmas are concerned in the pathogenesis of rheumatoid arthritis, it is highly unlikely that they would be eradicated by doses of tetracycline HCl as low as 250 mg. It is clear that doses far in excess of what could be tolerated in man would be required to produce a beneficial effect in rheumatoid patients. Drugs with much greater mycoplasmacidal action than the tetracyclines should be tried in rheumatoid patients before deciding that mycoplasmas cannot be causative agents in rheumatoid arthritis.

SUMMARY

Sodium aurothiomalate (ATM), gold keratinate and five different tetracyclines were investigated for activity against *M. arthritidis* strain ATCC 14124 and *M. pulmonis* strain JB, both *in vitro* and in rodents with arthritis caused by these mycoplasmas. *In vitro*, ATM had only slight activity against *M. arthritidis* and *M. pulmonis*, while gold keratinate was virtually inactive against *M. pulmonis*. In contrast, the tetracyclines were highly active against both mycoplasmas. The tetracyclines and the gold salts were both predominantly mycoplasmastatic.

In both rats and mice, parenteral administration of ATM, begun shortly before or after infection of the rodents with mycoplasmas, prevented the development of arthritis. ATM or gold keratinate, given subcutaneously to mice already arthritic from infection with *M. pulmonis*, reduced the severity of the arthritis, even although gold keratinate was inactive against this myco-

plasma *in vitro*. Moreover, direct testing of serum, collected from mice treated with gold keratinate, failed to demonstrate antimycoplasmal activity *in vitro*. These results suggest that the action of gold-containing drugs in mycoplasmal arthritis is due to biological properties of gold other than antimycoplasmal activity.

Tetracyclines were also found to be effective in preventing arthritis in rats and mice when given subcutaneously. With high doses, subcutaneous, but not oral, therapy significantly reduced the severity of established arthritis in mice infected with *M. pulmonis*. The blood levels achieved with the different tetracyclines, when related to their therapeutic activity, indicated that good antimycoplasmal activity and adequate absorption from the gut were not the only properties needed for optimal effectiveness. The results are discussed in relation to treatment of rheumatoid patients with tetracycline HCl.

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