

## Free thiomalate in plasma and urine of patients receiving sodium aurothiomalate

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**SUMMARY** Free thiomalate is measurable in the plasma and urine of patients with rheumatoid arthritis receiving sodium aurothiomalate (Myocrisin). Plasma concentrations and urinary excretion of free thiomalate were measured in 8 patients by high-performance liquid chromatography and electrochemical detection. After an initial intramuscular dose of 20 mg sodium aurothiomalate, free thiomalate was detected in plasma within 15 min, peak levels of up to 600 nmol/l occurring at 30 min. Plasma levels declined rapidly thereafter. During the first 24 h between 2 and 16% of the injected dose of aurothiomalate was recovered in the urine as free thiomalate, maximum excretion occurring during the first hour.

Although it is well recognised that only thiol-containing gold compounds act as effective anti-rheumatic agents,<sup>1</sup> the role of the thiol moiety in the activity of such compounds has been largely ignored. Jellum *et al.* suggested in 1977 that sodium aurothiomalate dissociates immediately following intramuscular injection to form free thiomalate and protein-bound gold.<sup>2</sup> Their initial experiments were carried out in animals with double isotope labelled aurothiomalate (<sup>198</sup>Au-<sup>14</sup>C) and showed differential excretion of the 2 radiolabels.<sup>3</sup> These experiments have recently been extended to man, but with only a single radiolabel (<sup>14</sup>C).<sup>4</sup> By means of gas chromatography and mass spectrometry Heininger *et al.* detected but did not quantitate free thiomalate in the urine of patients with rheumatoid arthritis receiving sodium aurothiomalate.<sup>5</sup> However, they were unable to demonstrate thiomalate in either serum or synovial fluid.

Thiol compounds have always proved difficult to quantitate in physiological fluids, because of both their inherent instability and the presence of endogenous thiols. The combination of high-performance liquid chromatography (HPLC) and electrochemical detection (ECD) has recently been shown to be a simple and rapid method for the detection of 2 thiol-containing drugs, D-penicillamine and captopril, in plasma and urine.<sup>6,7</sup> We have recently developed a similar method for the detection of free

thiomalate.<sup>8</sup> Here we describe the use of this technique to measure plasma and urinary levels of free thiomalate in 8 patients following their first injection of sodium aurothiomalate.

### Patients and methods

**Patients.** Eight patients (7 females, 1 male) were studied. Seven had definite or classical rheumatoid arthritis according to ARA criteria<sup>9</sup> and one had psoriatic arthritis. Their mean age was 45 years (range 28–67) with a mean disease duration of 3 years (range 1–6). None had previously received gold or D-penicillamine and none were currently receiving steroids. All were regularly taking nonsteroidal anti-inflammatory drugs.

**Sampling procedure.** After an overnight fast, control blood and urine samples were taken at 9.00 am (time 0). An intramuscular injection of 20 mg sodium aurothiomalate (Myocrisin) was then given. Blood samples were obtained by separate forearm venepuncture initially at 30, 60, 120, and 240 min; when the short half-life of plasma thiomalate became apparent, additional samples were taken at 15, 45, and 90 min. Samples were collected into tubes containing disodium ethylenediaminetetra-acetic acid (EDTA), final concentration 5 mmol/l, and centrifuged for 5 min. Plasma proteins were precipitated by the addition of 200  $\mu$ l of sulphosalicylic acid (2 mol/l) to 1 ml of plasma. After a further 5 min centrifugation the supernatant was rapidly frozen and stored at -20°C until analysis. Urine samples for the

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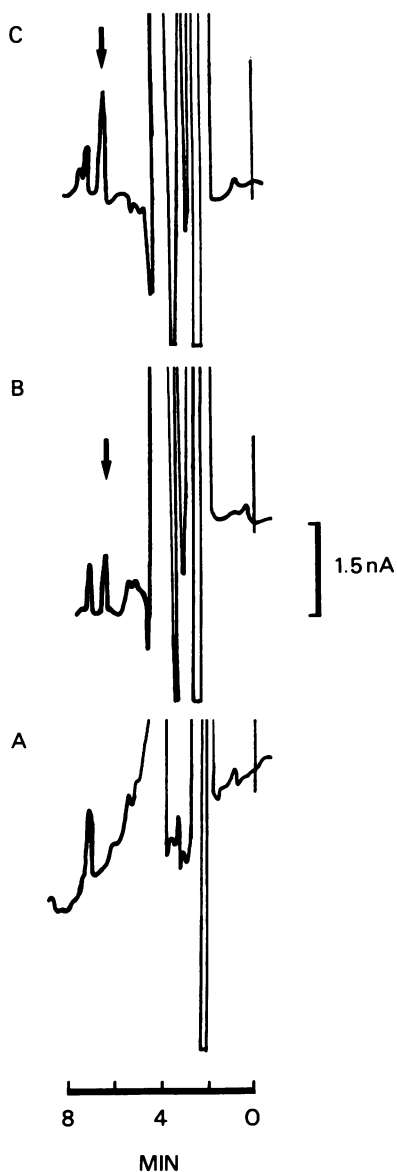


Fig. 1 Chromatograms showing the detection of thiomalate in plasma extract: A, before injection; B, 60 min following injection of 20 mg sodium aurothiomalate; C, as B but with added thiomalate. Broad arrows indicate thiomalate peaks.

periods 0–1 h, 1–2 h, 2–4 h, and 4–24 h were collected directly into containers containing 6 mol/l HCl (1 ml per 100 ml of urine). Urine volumes were measured and aliquots were then frozen at  $-20^{\circ}\text{C}$  until assay.

**Thiomalate assay.** Concentrations of free thiomalate were measured by HPLC and ECD at a gold electrode as previously described.<sup>8</sup> Briefly, the chromatographic system comprised an ACS 300/01 pump (Applied Chromatography Systems) and an EDT electrochemical detector LCA-15 (EDT Research) equipped with a LC-19 gold cell (Bio-Analytical Systems). The cell was maintained at +0.60 V for urine and +0.80 V for plasma, versus an Ag/AgCl reference electrode.<sup>8</sup> Separation of thiomalate from other sulphhydryl-containing compounds was performed on a 100 mm  $\times$  5 mm column packed with 3  $\mu\text{m}$  ODS Hypersil. The eluent consisted of a 0.1 mol/l aqueous phosphate buffer pH 2 containing 8% methanol for plasma samples and 1% methanol for urine samples. The detection limit for thiomalate in buffer using this technique was 40 fmol injected on to the column ( $S/N = 2$ ). Reproducibility was 2.1% ( $n = 10$ ), and the detector response was linear with increasing amounts of thiomalate injected ( $r = 0.99$ ,  $p < 0.001$ ). Recovery of thiomalate added to plasma at  $20^{\circ}\text{C}$  and immediately deproteinated was  $94.9 \pm 2.0\%$  ( $n = 5$ ). Recovery of thiomalate from urine was  $94.0 \pm 3.5\%$  ( $n = 6$ ).

**Statistics.** Data are presented as mean  $\pm$  1 SEM.

## Results

### PLASMA

Free thiomalate was detected in the plasma of seven of the 8 patients. Fig. 1 shows the presence of thiomalate in the plasma of a patient who had received 20 mg of sodium aurothiomalate by IM injection 60 min earlier; the concentration of thiomalate was 480 nmol/l. The thiomalate peak co-chromatographed with authentic thiomalate added to the same plasma (Fig. 1C).

Table 1 shows the plasma concentrations of free thiomalate in the 8 patients. Peak plasma levels were recorded between 30 and 45 minutes after injection of aurothiomalate and became undetectable by 4 hours. Mean concentrations are shown in Fig. 2.

### URINE

Free thiomalate was found in the urine of all 8 patients, with levels shown in Table 1; maximum excretion usually occurred during the first hour. Mean thiomalate excretion is shown in Fig. 3.

The percentage (in molar terms) of the injected dose of sodium aurothiomalate recovered from the urine as thiomalate within the 24 h after injection was calculated; this varied between 2 and 16% (Table 1).

## Discussion

In this study we have shown for the first time that free

Table 1 Plasma and urinary thiomalate in eight patients receiving sodium aurothiomalate

Patient	Plasma thiomalate concentration (nmol/l)								Excretion of urinary thiomalate ( $\mu\text{mol}$ )					Urinary recovery (%)
	Time after injection of aurothiomalate (min)								Period after injection of aurothiomalate (hours)					
	0	15	30	45	60	90	120	240	-0	0-1	1-2	2-4	4-24	Molar recovery of injected dose as thiomalate within 24 hours
1	0	*	UD	*	480	*	120	UD	0	4.28	0.43	0.43	UD	10.1
2	0	*	UD	*	UD	*	UD	UD	0	0.35	0.59	0.22	0.07	2.4
3	0	*	610	*	350	*	UD	UD	0	1.32	0.92	0.31	0.10	5.2
4	0	*	498	*	186	*	UD	UD	0	4.63	1.14	0.28	0.09	12.0
5	0	252	282	318	216	48	UD	UD	0	0.84	0.09	0.07	UD	7.8
6	0	UD	120	192	54	UD	UD	UD	0	2.80	0.86	0.37	UD	8.0
7	0	75	225	175	250	UD	UD	UD	0	4.21	2.50	1.74	UD	16.5
8	0	330	330	192	150	18	UD	UD	0	2.90	0.83	UD	UD	7.3

\*Not measured. UD = undetectable: detection limit for plasma is approximately 15 nmol/l.

Each patient received 20 mg sodium aurothiomalate ( $51.2 \mu\text{mol}$ ) by intramuscular injection at time 0.

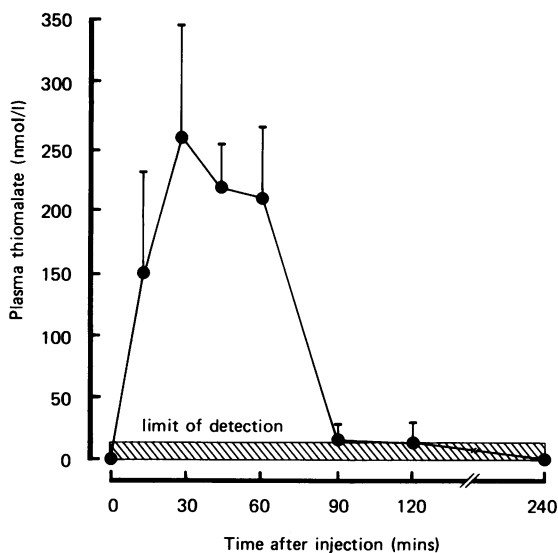


Fig. 2 Plasma concentrations of thiomalate following intramuscular injection of 20 mg sodium aurothiomalate in 8 patients. Data are presented as mean  $\pm$  SEM.

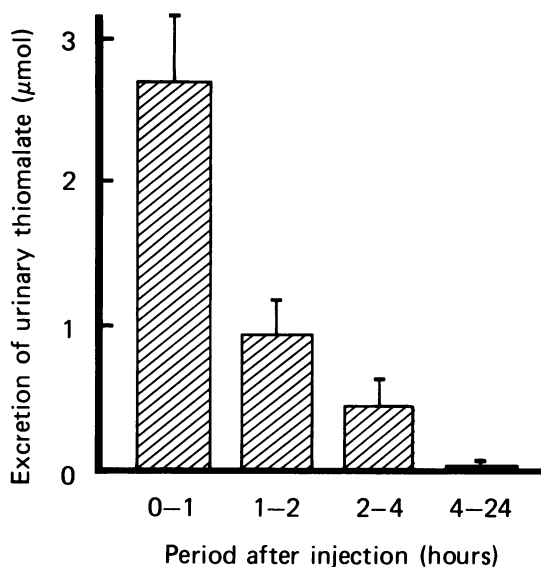


Fig. 3 Urinary excretion of thiomalate following injection of 20 mg sodium aurothiomalate. Data are presented as mean  $\pm$  SEM.

thiomalate circulates in human plasma after intramuscular injection of sodium aurothiomalate. Thiomalate may be detected as early as 15 minutes after injection; peak plasma levels are attained within 45 minutes and thereafter it rapidly becomes undetectable. It is also easily measurable in urine.

HPLC and ECD provides a simple, highly sensitive, and reproducible method for the detection and measurement of free thiomalate in physiological fluids. The plasma concentrations we have shown, even at peak levels, are well below the limit of detec-

tion of the assay techniques previously employed.<sup>5</sup> Even so we were unable to detect thiomalate in the plasma of patient 2. Since this patient's urinary excretion of thiomalate was the lowest recorded, plasma levels may have been below our limit of detection (approximately 15 nmol/l).

In their recent report Jellum and Munthe demonstrated the rapid initial urinary excretion of the carbon label of  $^{14}\text{C}$ -labelled sodium aurothiomalate.<sup>4</sup> After 24 h approximately 60% of the radioactivity had been recovered from the urine. The pattern of

urinary excretion of free thiomalate seen in our 8 patients closely resembles that found by these workers, though our percentage recovery is much lower. This is probably because this assay measures only free thiomalate whereas the  $^{14}\text{C}$  label employed by Jellum and Munthe, and identified by thin-layer chromatography, would represent both free thiomalate and its disulphide.

The similarity in chemical structure and clinical activity between thiomalic acid and several other thiol-containing second line antirheumatic agents, notably D-penicillamine, has been noted.<sup>1,2</sup> Animal studies by Arrigoni-Martelli *et al.*<sup>10</sup> and in-vitro work by Skosey and Chow<sup>11</sup> indicate that thiomalic acid may have potent antirheumatic properties, while human studies by Munthe and Jellum<sup>12</sup> suggest that oral sodium thiomalate without gold may be as effective as sodium aurothiomalate in the suppression of disease activity.

The demonstration of circulating free thiomalate in patients receiving intramuscular sodium aurothiomalate has several important implications. Firstly, it demonstrates that, as in patients receiving D-penicillamine, treatment with sodium aurothiomalate results in the circulation of a free thiol compound. Secondly, since thiomalic acid has been shown to possess antirheumatic properties, it suggests that some at least of the activity of sodium aurothiomalate may reside in its thiol moiety. Finally, the ability to measure plasma and urinary levels of this potentially active metabolite will be of considerable value in its further investigation.

Further work is necessary to define the metabolites of thiomalate; by analogy with D-penicillamine these would include the internal and mixed disulphides, which our present assay does not measure. For D-penicillamine these represent the most abundant circulating forms.<sup>13</sup> A controlled trial of sodium thiomalate without gold is also clearly indicated.

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