Distribution of gold among plasma fractions in rheumatoid patients undergoing chrysotherapy compared with its distribution in plasma incubated with aurothiomalate in vitro

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SUMMARY The distribution of gold among the globulin, albumin, and unbound fractions of plasma, obtained either from rheumatoid patients receiving long-term aurothiomalate therapy or from samples incubated with aurothiomalate in vitro, has been investigated. In the rheumatoid patients it has been found that, although the majority of the plasma gold is always bound to albumin, the distribution varies cyclically in phase with the dose schedule. An explanation of these phenomena is provided, based on data obtained from the reaction between aurothiomalate and plasma constituents in vitro.

Although sodium aurothiomalate (Myocrisin) and other gold-containing drugs are extensively used in the treatment of rheumatoid arthritis, relatively little is known about the mode of their pharmacological action. One of the most extensively investigated areas of gold metabolism has been concerned with its plasma kinetics. The main impetus behind such studies has been the desire to obtain a rational basis for designing efficient treatment regimens, by attempting to relate gold concentrations in easily obtainable body fluids, such as plasma, to phenomena such as therapeutic efficacy or toxicity. Although some workers have been able to relate high plasma gold levels to therapeutic efficacy (Lorber et al., 1973), toxic side effects (Kruzius et al., 1970), skin rashes (Jessop and Johns, 1973), and high urinary excretion (Billings et al., 1975), others have found no correlation between plasma gold concentrations and therapeutic response (Mascarenhas et al., 1972; Jessop and Johns, 1973; Gerber et al., 1972a, b), toxicity (Mascarenhas et al., 1972; Lorber et al., 1973), or tissue gold concentration (Palmer and Dunckley, 1973). Therefore the clinical value of monitoring total plasma gold levels is still conjectural.

Although the form in which a drug circulates invariably influences its biological effect, and for many drugs the active species has been shown to be that unbound (Goldstein, 1949), its applicability to aurothiomalate, or gold-containing drugs in general, has not been ascertained. The interaction between aurothiomalate and plasma or serum proteins has been studied both in vivo in rabbits (McQueen and Dykes, 1969) and rats (Lawson et al., 1977) and in vitro with human (Mascarenhas et al., 1972; Danpure, 1974, 1976a), rat, and fetal calf (Danpure, 1974) sera, but attempts to ascertain the distribution of gold in the plasma of rheumatoid patients receiving chrysotherapy have been scarce.

In the present investigation we have studied the distribution of gold in the various plasma fractions (as separated by gel chromatography) collected from 3 rheumatoid patients receiving long-term aurothiomalate therapy at various time intervals after the gold injection, and compared the results with those obtained by incubating gold-free human serum with aurothiomalate in vitro.

Methods

IN VIVO PLASMA BINDING Blood was obtained by venepuncture from three rheumatoid patients, receiving long-term chrysotherapy, at various times before and after an intramuscular injection of sodium aurothiomalate (Myocrisin) and was immediately placed on ice.
Distribution of gold

(These investigations were approved by the Ethical Committee of Northwick Park Hospital and the Clinical Research Centre.) Plasma was obtained from the heparinised samples by centrifugation at 1000 g for 5 min at 4°C. Within 10 min of the blood collection, 1 ml samples of plasma were layered onto columns (1·2 cm × 80 cm) of Sephadex G200 (Pharmacia GB Ltd., London), and eluted with 0·01 M Tris-HCl buffer pH 7·4 containing 0·15 M NaCl, 0·002 M sodium citrate and 0·005 % NaN₃ at 4°C. On the basis of the absorption profile at 280 nm the eluate was divided into globulin, albumin, and low-molecular-weight fractions (the latter eluting at the total column volume and representing non-protein bound material), freeze-dried, and redissolved in water to 10% of original eluate volume for that particular fraction. The gold concentration in the original plasma samples and the concentrated plasma fractions was determined by neutron activation analysis (Ward et al., 1977).

IN VITRO PLASMA BINDING

Sodium aurothiomalate (May and Baker Ltd., Dagenham, Essex), labelled with sodium (¹⁹⁵Au) aurothiomalate (Radiochemical Centre Ltd., Amersham, Bucks), was incubated at 37°C with fresh human plasma or serum (<30 min old). After various periods of time 400 µl was fractionated by chromatography through Sephadex G200 exactly as above. 4-ml fractions were collected, and their ¹⁹⁵Au content was determined by counting the gamma emission on a Wallac 300–1000 scintillation spectrometer.

Results

IN VIVO

The concentrations of gold in the plasma and its distribution in the plasma fractions from 3 rheumatoid patients receiving aurothiomalate therapy varied in a cyclic fashion and depended on the time after the previous injection, and, although quantitative differences occurred between the 3 patients, the results were qualitatively similar (Figs 1 and 2).

In all 3 patients the gold concentration in the whole plasma rose 5–10 times from the preinjection level (3 or 4 weeks after the previous injection) to reach a maximum after 100 min (Fig. 1). The level then fell slowly over the following weeks, so that by the time of the next injection (3 or 4 weeks) it had reached its original level.

The proportion of the gold in the whole plasma that was bound to the globulins decreased from 10 to 26% just before the injection to reach a trough...
(4–7%) 100–1000 min after injection (Fig. 2). On the other hand the concentration of gold bound to the globulins increased 2–3 times to reach a peak 30–100 min after injection, and although it decreased over the subsequent weeks it did not do so in any consistent fashion (Fig. 1).

The proportion of gold bound to albumin usually varied between 80 and 94% with a peak 100–300 min after injection (Fig. 2). However, in one patient (case 2) it dropped as low as 60%. Because the majority of gold in the plasma was bound to albumin, the changes in the concentration of gold in the albumin fraction closely paralleled those of gold in the total plasma (Fig. 1).

Although qualitatively similar, the greatest quantitative difference between the 3 patients occurred in the unbound gold profiles, the levels in 1 patient (case 2) being 3–5 times higher than those of the other 2. The proportion unbound decreased from 1.5 to 15% just before the injection to reach a trough of 0.6–1.0% after 100–300 min (Fig. 2). The concentration of unbound gold increased transiently 2–5 times to reach a maximum 10–30 min after injection (Fig. 1).

Attempts to determine the relationship between the distribution of gold within the plasma fractions and the concentration in the unfractionated plasma showed that, although the concentration of gold in the 3 plasma fractions in general correlated positively with the total plasma gold concentration (with varying degrees of significance), the proportion bound to albumin correlated positively ($r = +0.908$ to $+0.937$), while the proportion bound to the globulins or unbound correlated negatively ($r = -0.840$ to $-0.935$ and $-0.580$ to $-0.971$ respectively).

**IN VITRO**

The effects of aurothiomalate concentration and duration of incubation at $37^\circ$C on the distribution of gold among human plasma fractions in vitro are shown in Fig. 3. As has been shown previously (Danpure, 1976a), the rate of interaction between aurothiomalate and albumin was relatively slow, 100–300 min being required for the higher concentrations of aurothiomalate to reach a binding plateau, at which point 80-90% of the gold was albumin-bound. Because of the high affinity for aurothiomalate of the 1 interaction site on albumin (Danpure, 1976a), the proportion of gold bound to albumin remains steady at this fairly high level until the binding capacity of the albumin is exceeded (that is, at 500-700 $\mu$M aurothiomalate). On the other hand,
concentrations, whereas after the binding plateau had been reached (that is, 300 min) unbound gold was detectable only when the binding capacity of the albumin had been exceeded (that is, 500-700 μM). For example, when incubated with 10, 25, or 50 μM aurothiomalate (plasma concentrations frequently found in rheumatoid patients receiving chrysotherapy), 2.0%, 8.0%, or 15.0% respectively of the plasma gold was unbound after 10 min, 0.5%, 3.0%, or 7.0% after 30 min, and 0%, 0%, or 0.5% after 100 min.

**Discussion**

Most previous attempts to ascertain the in vivo distribution of gold in the plasma or serum of rheumatoid patients receiving aurothiomalate therapy have been qualitative or at best only semiquantitative. In the present study we have quantitated by neutron activation analysis the distribution of gold in the globulin, albumin, and unbound fractions, as separated by chromatography through Sephadex G200, from the plasma of 3 rheumatoid patients receiving long-term aurothiomalate therapy and compared it to the distribution of $^{195}$Au after incubating human serum with sodium $[^{195}$Au] aurothiomalate in vitro.

The results of the present study, which have confirmed that gold in the plasma of rheumatoid patients receiving aurothiomalate therapy is bound mainly to albumin (Fig. 1 and 2) as well as when human serum is incubated with aurothiomalate in vitro (Fig. 3), are compatible with those of previous studies. This has been shown to be the case with human serum in vitro (Mascarenhas et al., 1972; Danpure, 1974, 1976a), fetal calf serum in vitro (Danpure, 1974), rabbit plasma in vivo (McQueen and Dykes, 1969), and rat plasma or serum both in vitro (Danpure, 1974) and in vivo (Lawson et al., 1977). In addition Eberl and Altman (1970) found that most of the plasma gold was bound to albumin in vivo in rheumatoid patients receiving aurothiogalactosyl sulphate (Sanocrysin). It has been shown previously (Danpure, 1976a) that, although aurothiomalate reacts slowly with albumin in fresh human serum, the gold-albumin complexes formed are very stable. The reaction probably involves the free thiol of mercaptalbumin (Gerber, 1974; Danpure, 1976a, b) in the first instance and possibly the masked disulphide derivative (Danpure, 1976a, 1977a; Danpure and Lawson, 1977) subsequently, yielding a total of 1 gold-binding site per albumin molecule (Danpure, 1976a).

In the present study it was found that, both in vivo and in vitro, most of the plasma gold that was not bound to albumin was bound to the globulins

![Graph](http://ard.bmj.com/...)

**Fig. 3** Percentage distribution of $^{195}$Au in the globulin (A), albumin (B), and unbound (C) fractions from human plasma after incubation at 37°C for 10 min (□ □ □), 30 min (Δ △ △), 100 min (O — — — O), or 500 min or more (● — — — ●). Albumin and globulin concentrations were 40-46 g l$^{-1}$ (590-680 μM) and 20-25 g l$^{-1}$ respectively.
except in vitro at very high aurothiomalate concentrations or very short incubation times). Few other data exist on the binding of gold to globulins. Mascarenhas et al. (1972) found that the globulins of human plasma bound gold when incubated with aurothiomalate in vitro, as did McQueen and Dykes (1969) with rabbit plasma and Lawson et al. (1977) with rat plasma in vivo. In a semiquantitative investigation in vitro, Lorber et al. (1972) found that increasing the concentration of aurothiomalate from 5 to 35 μM (100 to 700 μg Au 100mL⁻¹) caused increased binding of the gold to the globulins of rheumatoid sera. In the present study, when serum was incubated for 16 hours with increasing concentrations of aurothiomalate (10–1000 μM), the concentration of gold bound to the globulins increased approximately proportionately, so that the percentage bound remained fairly constant between 10 and 23%. On the other hand, in the plasma of rheumatoid patients in vivo, as well as in the plasma of rats in vivo (Lawson et al., 1977) there was a distinct inverse correlation between the total plasma gold concentration and the proportion of the gold bound to the globulins (even though the concentration of gold bound to the globulins showed a slight positive correlation).

The interactions between aurothiomalate and albumin or the globulins appear to be quite different in a number of respects. Although the amount of gold normally bound to albumin is much greater than that bound to the globulins (80–90% compared to 10–20%), the rate of reaction with the latter appears to be much faster than with the former. For example, when serum was incubated with 100 μM aurothiomalate (Fig. 3), the binding plateau for albumin was not reached until 5 hours, whereas the binding to the globulins was completed within 3 min. In addition, the maximum binding capacity of the globulins for gold appears to be in excess of that of albumin, as demonstrated by the fact that even up to 2500 μM aurothiomalate no binding plateau was observed, whereas the binding capacity of albumin was exceeded at an aurothiomalate concentration of 500–700 μM (as albumin possesses 1 interaction site per molecule [Danpure, 1976a]).

The stabilities of the gold-albumin complexes resulting from the interaction with aurothiomalate are such that after the attainment of equilibrium (that is, at 5 hours or more) no unbound gold was detectable unless the aurothiomalate was added in molar excess (that is, about 500–700 μM). This concentration is 10–20 times the peak normally encountered in the plasma of rheumatoid patients receiving chrysotherapy. Therefore one would normally not expect to be able to detect any unbound gold at therapeutic plasma concentrations in vivo. Indeed, in the present study very little unbound gold was found from 5 hours after injection onwards.

However, the reaction between aurothiomalate and albumin was slow, so that even at fairly low concentrations of aurothiomalate unbound drug could be detected after short periods of incubation. Similarly in rheumatoid patients receiving chrysotherapy small transient peaks of unbound gold, presumably aurothiomalate, could be detected shortly (10–30 min) after injection (Fig. 1).

Previous investigations into the levels of unbound gold in the plasma of rheumatoid patients receiving aurothiomalate therapy have been somewhat contradictory. In an abstract Campion et al. (1974) reported having found 8–10% unbound gold 1, 2, or 4 weeks after aurothiomalate, while the serum levels were 6·3–17·5 μg atom 1⁻¹ (124–349 μg Au 100 ml⁻¹). On the other hand Kamel et al. (1976) were unable to detect any unbound gold in 1 plasma sample from a rheumatoid patient receiving chrysotherapy. It is difficult to compare these results with those of the present study, as both Campion et al. (1974) and Kamel et al. (1976) used ultrafiltration, a technique which is highly susceptible to the contamination of the filtrate with small quantities of serum proteins. In addition they did not appear to take the precaution of minimising further interaction between the aurothiomalate and proteins after sample collection by decreasing the temperature and fractionating immediately.

Although the profile of unbound gold was qualitatively similar in the 3 patients of the present study, they showed marked quantitative differences. The reason for this is unknown but may indicate different rates of interaction between aurothiomalate and albumin. Nevertheless, the interaction between aurothiomalate and plasma proteins, especially albumin, was enough to dampen considerably in all 3 patients any possible fluctuations in the concentration of unbound drug in the plasma as a result of a large influx of newly administered aurothiomalate.

The rate of interaction between aurothiomalate and albumin in vivo might be expected to play a key role in the size and duration of any peak of unbound aurothiomalate. In this respect it is interesting to note that the rate of interaction between aurothiomalate and albumin in fresh rat serum appears to be faster than with human serum (Danpure, 1974), and in addition no transient peak of unbound gold can be detected in rat plasma in vivo, even at total plasma gold concentrations of 10 times those found in humans (Lawson et al., 1977).

Although the transient peak of unbound gold (aurothiomalate ?) is small (relative to the total plasma concentration) and very variable, it may play an important role in the tissue uptake and excretion...
of gold. Freyberg et al. (1941), Sliwinski et al. (1966), and Mascarenhas et al. (1972) showed that the urine content of gold rose sharply soon after the injection of the gold drugs, this rise implying the presence of unbound drug in the plasma.

Although the baseline levels of gold in 2 of the patients (cases 1 and 3) were extremely low and hardly detectable, in the third (case 2) they were much higher and 4 weeks after the previous injection accounted for as much as 15% of the total plasma gold. This is far in excess of that expected if the unbound gold was in the form of unaltered aurothiomalate.

It has been shown that aurothiomalate can react with low-molecular-weight thiols, such as cysteine (Danpure, 1976b), and disulphides, such as cystine (Danpure and Lawson, 1977), under appropriate conditions forming S-aurocysteine. However, the products of such reactions can be complex in nature (Isab and Sadler, 1976) and may play an important role in the metabolism of aurothiomalate. Chronically gold may be released back into the blood from the tissues in the form of such complexes; these may be manifested by the baseline levels of unbound plasma gold (especially in case 2) found in the present study. In this particular patient at any rate the gold in the transient peak (aurothiomalate ?) might be quite different from that constituting the baseline levels (Danpure, 1976b). As a result it might be profitable to investigate the chemical nature of the gold excreted in the urine soon after injection and at longer time intervals.

Successful management of the treatment of rheumatoid arthritis by gold drugs has been plagued by the inability consistently to relate any easily measurable parameter to potential efficacy or toxicity of the treatment. As the metabolism of many drugs is influenced by the form in which they circulate in the blood (Goldstein, 1949), it is possible that measurement of parameters such as those investigated in the present study (especially the unbound forms) might provide a clearer understanding of what influences the outcome of a particular schedule of gold therapy. Howell et al. (1975) have shown that the bone marrow from patients having previously suffered from gold-induced agranulocytosis was not abnormally sensitive to aurothiomalate in vitro. Therefore they suggested that in these patients aurothiomalate might be metabolised differently from how it is in patients that did not suffer bone-marrow depression. The 3 patients monitored in the present study, despite being qualitatively similar, showed marked quantitative differences (especially of the unbound form), indicating that different people may metabolise aurothiomalate differently. However, further work is necessary before delineation of the exact relationship between the form of plasma gold and therapeutic outcome can be achieved.

References


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