Gold levels produced by treatment with auranofin and sodium aurothiomalate

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SUMMARY Sixty-three patients with rheumatoid arthritis were randomly divided into 3 groups, and treated with either sodium aurothiomalate (Myocrisin), auranofin, or placebo. Gold levels in whole blood, plasma, and haemolysate were measured serially along with clinical and laboratory parameters of efficacy. Auranofin produced a higher ratio of haemolysate to plasma gold than Myocrisin, and it appears that the affinity of the red cell for gold is reduced during therapy with auranofin. Gold levels did not correlate with changes in the pain score, erythrocyte sedimentation rate, and C-reactive protein, nor with the development of toxicity. In the Myocrisin group the haemolysate gold level achieved was dependent on the number of cigarettes smoked. In the auranofin group there was no such correlation, but the haemolysate gold level was higher for smokers than non-smokers. The likely action of gold is discussed.

Gold compounds have been used in the treatment of rheumatoid arthritis for over 50 years, and their place in clinical practice is firmly established.^{1,2} However, there remains a need to monitor the use of these compounds very carefully to establish efficacy and to anticipate the onset of any toxic reaction which may occur. At present the mechanism of action of these compounds is unknown, and no specific parameter, such as the plasma gold level, has been found to provide an early indication of either efficacy or toxicity.

Gold is usually administered parenterally as Myocrisin (sodium aurothiomalate), but a new orally administered and chemically different compound, auranofin (triethylphosphine gold thioglucose tetraacetate or triethylphosphine gold-2,3,4,6-tetra-*o*-acetyl-1-thio- β -D-glucopyranoside) has become available for testing and controlled clinical trials.³ In animal studies the distribution of gold produced by the 2 compounds is different, with auranofin producing lower tissue gold levels and a higher erythrocyte gold level.^{3,4} It has also been suggested that the erythrocyte gold level is a better indicator of tissue gold levels during Myocrisin therapy⁵ and that the erythrocyte gold level is a function of cigarette smoking.⁶

The purpose of the present study was to measure the distribution of gold in the blood of patients receiving Myocrisin or auranofin, both in order to determine whether or not parameters such as haemolysate gold or the ratio of haemolysate to plasma gold are correlated with efficacy or toxicity and to improve our understanding of the in-vivo chemistry of gold drugs.

Materials and methods

Sixty-three patients with definite or classical rheumatoid arthritis, according to the criteria of the American Rheumatology Association, were included in the study. All had active synovitis unresponsive to nonsteroidal anti-inflammatory drugs. They had not previously been treated with gold in any form and had not received penicillamine, levamisole, immunosuppressive drugs, or corticosteroids in the 3 months preceding this trial.

The patients were randomly allocated to 3 groups. Twenty-one patients (4 male and 17 female, median age 46 years, range 28-69) were given weekly intramuscular injections of 50 mg of Myocrisin for 12 weeks following an initial test injection of 10 mg. Thereafter the frequency of injection was varied from 1 to 4 weeks according to clinical response. Twenty-one patients (5 male and 16 female, median age 57 years, range 28-71) received auranofin 3 mg

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b.d. throughout the study. Twenty-one patients (5 male and 16 female, median age 55 years, range 31–72) received placebo tablets identical with auranofin twice daily.

Blood was taken by venesection prior to the next gold or placebo dose at 0, 3, 6, 12, and 24 weeks. 5 ml of the heparinised blood was centrifuged at 3000 g for 10 minutes, and the plasma was removed by suction. The cells were washed twice with isotonic saline solution and lysed with an equal volume of distilled water (2 h at 4°C). The sample was centrifuged again (3000 g for 10 min) and a sample of haemolysate removed by suction. Whole blood, plasma, and blood levels of haemolysate gold were determined by diluting the samples 15 times with distilled water and analysing for gold by atomic absorption spectrometry using carbon furnace atomisation. The methodology has been published previously. Care should be taken to ensure that the background corrector is working satisfactorily and that all sources of smoke and saline interference have been successfully eliminated.⁷

Clinical and other biochemical assessments of disease activity were performed weekly or monthly. Pain score, visual analogy scale, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) values were selected as the most useful measurements to compare with gold levels.

The statistical comparison was by Wilcoxon's matched-pairs signed-rank test or linear regression as appropriate.

Results

Both Myocrisin and auranofin produced a clinical improvement as measured by falls in ESR and CRP between zero and 24 weeks. However, Myocrisin produced an earlier response which was significant at 12 weeks and produced an improvement in pain score which appears to be sustained through the 24-week period (Table 1). Of the 63 patients 48 completed the 6-month study. The largest drop-out occurred in the placebo group, due to the lack of effect. Only a few patients discontinued active

Table 2 Gold levels of patients withdrawing from the trial

Treatment	Week	Reason for withdrawal	Plasma Au (µg/ml)	Haemolysate Au (µg/ml)
Myocrisin	1	Nitritoid reaction	—	—
	16	Rash	1.9	0.2
	16	Rash	2.0	0.2
	20	Rash	2.7	1.0
	20	Proteinuria	5.2	0.1
Auranofin	5	Diarrhoea	0.2	0.5
	6	Leucopenia	0.1	—
	16	Haematuria	1.1	1.0
Placebo	3	Diarrhoea	—	—
	8	Haematuria	—	—
	12	7 patients—lack of effect	—	—

SI conversion: µg/ml = mg/l.

Fig. 1 Plasma and lysate gold levels following administration of Myocrisin or auranofin. Lysate values are shaded. The heights of the bars are medians and the error bars refer to interquartile ranges.

Table 1 A statistical analysis of the change in pain score, ESR, and CRP measurements between week 0 and weeks 12 and 24 of the trial. Wilcoxon's matched-pairs signed-rank test was used

Compound	Week	Pain score	ESR	CRP
Auranofin	12	p<0.05	NS	NS
	24	NS	p<0.05	p<0.01
Myocrisin	12	p<0.05	p<0.01	p<0.01
	24	p<0.01	p<0.01	p<0.01
Placebo	12	p<0.05	NS	NS
	24	NS	NS	NS

therapy. There was no obvious correlation between plasma or lysate gold levels and drop-out due to rash or other toxic reaction with either Myocrisin or auranofin. However, the number of drop-outs was rather small (Table 2).

Plasma gold levels increased with time in both Myocrisin and auranofin groups (Fig. 1). The Myocrisin group reached a plateau value more quickly at about 6 weeks, whereas with auranofin a plateau value was reached after about 13 weeks. The 24-week result reflects maintenance therapy with Myocrisin and continuing therapy with auranofin. The median gold concentration between 6 and 12 weeks with Myocrisin was 3.6 $\mu\text{g/ml}$ and with auranofin was 0.9 $\mu\text{g/ml}$. The interquartile range is larger with auranofin. (SI conversion: $\mu\text{g/ml} = \text{mg/l}$.)

Haemolysate gold levels were detectable during both Myocrisin and auranofin treatment. Haemolysate gold concentrations were lower with Myocrisin than auranofin (median concentrations after 12 weeks of therapy were 0.66 $\mu\text{g/ml}$ and 0.95 $\mu\text{g/ml}$, respectively). Since plasma gold levels were much higher with Myocrisin, the haemolysate-to-plasma ratio was much lower. After 3 weeks of auranofin therapy a very high haemolysate-to-plasma ratio was recorded, but with that exception this ratio remained approximately constant with time for each drug.

To examine the reactivity of the gold administered orally, separated plasma samples from an auranofin-treated patient and from a Myocrisin-treated patient after 12 weeks' therapy were incubated for 12 hours with separate aliquots of placebo cells. The cells were separated and a haemolysate prepared as before. About 10 to 20% of the Myocrisin gold was transferred to the placebo cells and most of the auranofin plasma gold was transferred (Table 3). Thus, there

Table 3 Gold incubation study. Erythrocyte and plasma gold levels measured on 3 individual patients after 12 weeks of treatment. Plasma and cells were then exchanged, incubated for 12 hours, re-separated, and gold levels remeasured. (a) Gold levels in each component at the start of experiment; (b) gold-containing cells and placebo plasma; (c) placebo cells and gold-containing plasma

Cells	Plasma	Gold levels (in $\mu\text{g/ml}$)	
		Cell	Plasma
(a) Auranofin	Auranofin	0.99	0.79
Myocrisin	Myocrisin	0.3	3.36
Placebo	Placebo	0.0	0.0
(b) Auranofin	Placebo	0.72	0.0
Myocrisin	Placebo	0.28	0.0
(c) Placebo	Auranofin	0.68	0.05
Placebo	Myocrisin	0.05	3.0

SI conversion: $\mu\text{g/ml} = \text{mg/l}$.

was a different affinity for the plasma gold in its auranofin-administered form than for Myocrisin, and untreated cells appeared to be able to absorb gold not taken up by the original auranofin-treated cells. No appreciable amount of gold was transferred from either auranofin- or Myocrisin-treated cells to placebo plasma.

Although there was a continual increase in gold level on both treatments, there was no correlation between clinical improvement as measured by ESR or CRP and the gold level for whole blood, plasma, haemolysate, or haemolysate-to-plasma ratio, suggesting that none of these parameters may be used *per se* as an indicator of clinical or toxicological response.

Cigarette smoking affected the haemolysate-to-plasma ratio with both auranofin and Myocrisin (Fig. 2), but the ratio for non-smoking patients treated with auranofin was sufficiently high to suggest that there was a non-smoking-dependent mechanism of absorption in that case. A plot of the number of cigarettes against haemolysate gold for Myocrisin-treated patients who smoke indicated that there was a statistically significant ($r = 0.86$, $p < 0.001$) dose-response relationship (Fig. 3). There was no similar correlation with auranofin.

Fig. 2 A comparison of the ratios of haemolysate-to-plasma gold for auranofin smokers and non-smokers with those for Myocrisin smokers and non-smokers.

Fig. 3 A relationship between the number of cigarettes smoked and gold level for patients after 12 weeks of Myocrisin therapy.

Discussion

Modern gold therapy uses a gold(1)-thiol compound such as Myocrisin, Solganal (aurothioglucose), or auranofin. In each of these compounds the gold is tightly bound to the sulphur, and in the case of auranofin it is also bound to a phosphorus group.⁴ In vivo it seems likely that there will be an exchange between the sulphur and phosphorus ligands and naturally occurring thiols as

and it is believed that the in-vitro forms of gold consist of a series of such compounds, with little or no free gold. It has been suggested that the thiol or sulphhydryl group ligand is the active species,⁸ but the quantities used in gold therapy are much smaller than those used with active ligands containing thiols (for example, penicillamine), so that it seems more likely that it is the gold moiety which is the active constituent.

Auranofin is a rather different compound from Myocrisin in that it is more lipid and less water-soluble, and it is monomeric whereas Myocrisin is polymeric. One objective of the present study was to try to discover whether a common form of gold was produced in serum. The experiment in which placebo cells were incubated with the plasma confirmed what is clear from the distribution, that the forms of gold in the plasma after 12 weeks are chemically distinct.

A higher proportion of the auranofin gold is in the erythrocytes 3 weeks after the first administration of gold than at longer time intervals. Since virtually all plasma gold after 12 weeks of therapy can still be removed by placebo erythrocytes, it would seem that the nature of the erythrocyte alters during therapy

with auranofin, either because the membrane changes or because some saturation process occurs.

With auranofin there appears to be a natural mechanism of cellular uptake which is missing with Myocrisin. This could be due, for example, to extra negative charges on Myocrisin metabolites, to their polymeric nature, or to their affinity for aqueous rather than lipid media. In smokers either the cell membrane is disturbed or, more likely, the extra thiocyanate or cyanide ligands added to the plasma react with the gold to form a compound that is readily transported across the membrane. Thiocyanate is present in the larger amounts in the blood of smokers⁹ but is the least likely of the 2 ligands to be active, since gold thiocyanate is not very stable and is unlikely to exist in a thiol matrix such as blood and tissue. It seems more likely that cyanide will form a stable complex which can be transported across the membrane. The cyanide may then recycle and pick up more gold and so could act as an effective transporting agent in the quantities available in-vivo.

Thus 2 quite different compounds appear to form different metabolites and give different gold distributions in blood. Nevertheless, both produce a therapeutic response which is likely to be due to the gold moiety. Plasma and lysate gold levels do not correlate with therapeutic response and there seems no evidence that smoking enhances the action of Myocrisin, so that the measurement of intracellular or extracellular gold levels does not seem to be useful in monitoring gold therapy per se. The conventional explanation for the action of gold, namely, that it stabilises lysosomal membranes, is based on the detection of electron-dense deposits¹⁰ round the membrane and on the discovery of aurosomes in white cells.¹¹ This form of gold accounts for very little

of the total body gold, and the techniques used are insufficiently sensitive to pick up the reactive molecular gold in the system. In view of the apparent effect of auranofin on cell membranes, and since intracellular parameters are not more, and are possibly less, significant than extracellular ones, it seems possible that the true role of gold is to modify some sulphhydryl-disulphide interchange processes by reacting with them, possibly at the cell membrane. Thus a more germane approach to the problem of predicting efficacy and toxicity may be to investigate the effect of plasma gold on the cellular function of leucocytes. In any event auranofin seems to be effective, albeit at a slower rate, at gold concentrations in blood which are below those used in Myocrisin therapy, and it may be possible to maintain patients on this compound at a full dose for a much longer period than with Myocrisin.

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