The Role of the Ligand in Chrysotherapy: a Kinetic Study of ^{199}Au- and ^{35}S-Labelled Myocrisin† and Auranofin†

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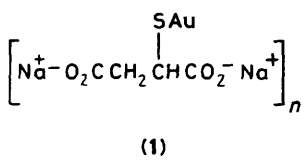
The distribution of gold and sulphur in blood, skin, bone, joints, and all major organs of rats has been monitored over 7 days following administration of a single dose of radiolabelled (^{199}Au or ^{35}S) gold sodium thiomalate (Myocrisin, 2.5 mg kg^{-1}, 6.4 μmol kg^{-1}, i.m.) or triethylphosphinegold tetra-acetylthioglucose (Auranofin, 2.5 mg kg^{-1}, 4.0 μmol kg^{-1}, p.o.). A major difference from previous studies is the effectively complete absorption of the administered Auranofin; this is attributed to its administration as a solution (5% ethanol–water) rather than as the solid. Even so, absorption of gold from Auranofin is relatively slow but, after 24 h, the pattern of distribution is broadly the same for the two drugs, the level from Auranofin being slightly the lower. The distribution of sulphur is very different; in particular, the amounts of sulphur in every organ are considerably less than those of gold. This indicates cleavage of the Au–S bond early in the metabolic process, probably in the stomach or intestine. Differences in the rate of clearance of Myocrisin- and Auranofin-gold from the blood indicate that at least some of the gold is present in different chemical forms for the two drugs. The data are discussed in terms of the known *in vitro* chemistry.

Thiolate derivatives of gold, AuSR, have been used for several decades as effective anti-arthritic agents.[1–3] Those most commonly used are gold sodium thiomalate [(1), GST, Myocrisin] and the corresponding glucose thiolate [(2), Solganol]. These materials are water soluble, and are normally administered by intramuscular (i.m.) injection. The recently introduced compound Auranofin [(3), AF, Et_3PAuSR, HSR = 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose] has different



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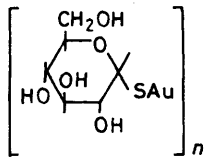

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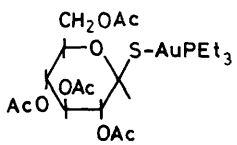


(3)

solubility characteristics, and can be given by mouth (p.o.). The two drugs have rather different clinical and toxic characteristics,[4–8] and appear to lead to different retention of gold, especially in the kidney.[6–9]

It is widely believed that the beneficial effects of these drugs is due to their gold content.[10,11] However, it has been pointed out that metabolic reactions lead to release of the thiol and, since other thiols, such as penicillamine, are effective, it may be the released thiol which is the active component.[12–16] While many studies have been made of the kinetics of distribution and elimination of gold from GST (see Table 1),[6,13,17,18] there are only two detailed reports of the thiolate distribution,[10,14] which show poor agreement (Table 2). For AF, there are a few reports on gold distribution[18–22] (see Table 1), but very little information on the thiolate.[18] However, the gold-distribution data suggest that there is lower retention by organs, such as the kidney, of gold from AF than from GST,[6,9] raising the possibility of lessened renal toxicity. If this difference is real, it is presumably due to the difference in initial ligation of the gold which affects its metabolism. Detailed distribution data for the metal and for the ligand are thus an essential preliminary for an understanding of the biochemistry of the drugs.

We have therefore undertaken a study of the kinetics of distribution of ^{199}Au- and ^{35}S-labelled GST and AF in rats.

Experimental

All preparations were tested with inactive materials and the products were characterized by analysis (Table 3) and t.l.c. (visualization by iodine vapour, single spots in all cases, solvents as described below).

Preparation and Separation of Gold-199.—Platinum sponge of natural isotopic composition (500 mg) was irradiated in the Manchester and Liverpool Universities' Research reactor for 8 h at a flux of *ca.* 3 × 10^{12} n cm^{-2} s^{-1}, allowed to decay for 16 h, and dissolved in *aqua regia*. The solution was evaporated almost to dryness and the residue was heated twice with a few drops of concentrated hydrochloric acid to remove nitric acid and oxides of nitrogen. The final residue was dissolved in hydrochloric acid (2 mol dm^{-3}, 2.0 cm^3) and the gold-199 was extracted by shaking with ethyl acetate. Gamma-ray analysis showed that four extractions gave complete recovery of the gold. Traces of platinum transferred to the organic phase were removed by back-washing it with hydrochloric acid (2 mol dm^{-3}). The gamma-ray spectrum of the gold solution showed

† Disodium [mercaptosuccinato(3−)-*S*]aurate(I), and (2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)(triethylphosphine)gold(I), respectively.

Table 1. Gold distribution following a single dose of Myocrisin or Auranofin

Animal	Dose/mg(Au) kg^{-1}	t/h	Organ level/%dose g^{-1} Blood	Kidney	Spleen	Liver	Skin	Muscle	Excreted	Ref.
Myocrisin										
Mice	0.5	6	7.9	8.9	1.7	3.2	1.8	0.9	2.3	13
		20	4.4	17.1	1.8	2.5	2.1	0.6	19	13
Rats	4.1	72	0.25	0.15	0.10	0.10	0.04	0.2	33	17
Auranofin										
Rats	0.29[a]	24	0.38	2.3	0.31	0.11		0.06		18
		120	0.04	2.2	0.25	0.11		0.03	86[b]	6

[a] Calculated assuming animals weighed 150 g. [b] After 3 days.

Table 2. Thiolate distribution following a single dose of Myocrisin

Animal	Dose/mg(Au) kg^{-1}	t/h	Organ level/%dose g^{-1} Blood	Kidney	Spleen	Liver	Skin	Muscle	Excreted	Exhaled	Ref.
Mice	1.0	6	0.1	1.9	1.3	1.8	2.6	2.4	2.3	0.4	10
		20	0.1	3.1	1.1	1.3	0.8	0.6	46	35	10
Rats	8.2	72	0.055	0.019	0.010	0.013	0.004	0.048	82	14	14

Table 3. Analytical data

Compound[a]	%C	%H	Other			
A	37.3(37.0)	5.0(4.8)	N	5.7(5.7)	S	6.7(6.6)
B	41.0(41.0)	4.7(4.6)	Br	18.1(19.5)		
[AuCl(PEt_3)]	20.5(20.5)	4.6(4.3)	P	8.8(8.8)		
AF	34.5(35.3)	5.3(5.1)	P	4.8(4.6)		
GHT	13.4(13.8)	1.7(1.5)	S	9.3(9.3)		

[a] **A** = *S*-(2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl)thiopseudourea hydrobromide; **B** = 2,3,4,6-tetra-*O*-acetylglucopyranosyl bromide; GHT = gold thiomalic acid, $AuSCH(CO_2H)CH_2CO_2H$.

trace amounts (*ca.* 0.25%) of gold-198; since the quantity was so low and its half-life (2.7 days) is similar to that of gold-199 (3.14 days), its presence was ignored. The overall yield was *ca.* 6 μCi of gold-199 per milligram of natural platinum, obtained as $HAuCl_4$ in ethyl acetate.

[^{199}Au]GST.—An ethyl acetate solution obtained as above (1.05 mCi of gold-199) was diluted with inactive chloroauric acid (52 mg, 0.13 mmol) in ethyl acetate (2.0 cm^3). Aqueous sodium cyanide (2 mol dm^{-3}) was added dropwise with continuous stirring until the organic layer became colourless. Hydrochloric acid (2 mol dm^{-3}) was then added, the mixture was stirred for a few minutes, and the two layers were separated. The ethyl acetate layer was evaporated to dryness, and the white residue was heated at 110 °C until a uniform yellow colour developed (*ca.* 30 min). [CARE: CYANOGEN IS PRODUCED IN THIS REACTION.] The solid gold(I) cyanide thus obtained was washed with water, suspended in water (2.0 cm^3), and treated with an aqueous solution of thiomalic acid (30 mg, 0.2 mmol). The mixture was stirred and heated on a water-bath until the solid had completely dissolved (2—3 h), and the resulting solution was filtered and evaporated to dryness. The off-white, sticky residue was triturated with ethyl acetate to remove any excess of thiomalic acid, dried, and dissolved in water (1.0 cm^3). After neutralization to litmus with sodium hydroxide (2M), the solution was ready for dilution and injection (yield 31 mg, 0.08 mmol; 750 μCi: 70%). Purity was checked by t.l.c. on alumina–glass plates with isopropyl alcohol–water (7:3) and by paper chromatography in water.

^{199}[Au]AF.—Triethylphosphinegold-199 chloride was prepared by the method of Hill and Sutton.[23] An aqueous solution of chloroauric acid was reduced with thiodiglycol[24] and treated with triethylphosphine. The separated gold complex (30 mg, 85 μmol) was dissolved in ethanol (2.0 cm^3) and treated with a suspension of silver acetate (25 mg, 162 μmol) in ethanol (2.0 cm^3). The mixture was stirred at 0 °C in the dark for 4 h and filtered, and a few drops of dichloromethane were added. The thioglucose was prepared by the addition of a solution of potassium carbonate (117 mg, 85 μmol) in water (2.0 cm^3) to a solution of *S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thiopseudourea hydrobromide (**A**: 41 mg, 85 μmol) in water (3.0 cm^3) at 0 °C. The cold solution of the gold complex was immediately added, and the mixture was stirred at 0 °C for 1 h. Concentration to half volume and cooling of the mixture gave [^{199}Au]AF (43 mg, 74%), which was further purified by dissolution at room temperature in the minimum amount of methanol, adding water until the mixture became cloudy, and cooling. The yield at this stage was 38 mg (66%). The purity of the product was checked by t.l.c. on silica–glass plates with acetone–dichloromethane (1:19), alongside an authentic sample.

[^{35}S]GST.—[^{35}S]Thiomalic acid was synthesized by addition of an aqueous solution (3.0 cm^3) of sodium [^{35}S]sulphide (205.5 mg, 2.6 mmol, 2.0 mCi) to an aqueous solution (14 cm^3) of bromosuccinic acid (0.5 g, 2.5 mmol) at pH 7.2. The mixture was sealed and stirred at 45—55 °C for 3 days, during which time the pH fell to *ca.* 5.5. The mixture was cooled to room temperature, acidified (H_2SO_4) to pH 1.5, and extracted with diethyl ether. Removal of the solvent gave a solid (46.4 mg), which was used without further purification by dissolution in water (10 cm^3) and treatment with gold(I) cyanide (66.9 mg, 0.30 mmol). After having been stirred at 95 °C for 3 h, the

Table 4. Distribution of GST-gold and AF-gold (as ^{199}Au, percentage of initial dose per gram) at various times after administration[a]

Organ	2 h	5 h	24 h	48 h	168 h	Drug
Blood	2.7(3)		1.2(2)	0.74(1)	0.086(29)	GST
	0.93(13)	1.3(2)	1.8(1)	0.83(3)	0.076(1)	AF
Kidney	6.4(10)		8.4(5)	9.3(18)	12(9)	GST
	1.1(1)	3.1(6)	6.8(9)	7.1(10)	6.6(7)	AF
Liver	0.83(17)		0.78(10)	0.70(12)	0.60(12)	GST
	0.34(6)	0.63(11)	0.65(6)	0.32(6)	0.30(4)	AF
Spleen	0.80(8)		1.3(2)	1.6(19)	1.6(2)	GST
	0.28(4)	0.58(8)	0.99(16)	0.69(17)	0.53(10)	AF
Lung	1.52(120)		1.18(11)	0.806(52)	0.646(103)	GST
	0.38(6)	0.60(16)	0.92(2)	0.51(6)	0.21(2)	AF
Joint	0.40(5)		0.35(4)	0.34(3)	0.28(3)	GST
	0.12(2)	0.24(3)	0.45(7)	0.37(5)	0.30(5)	AF
Bone	0.24(4)		0.22(8)	0.18(3)	0.10(1)	GST
	0.11(1)	0.15(2)	0.31(4)	0.21(4)	0.14(4)	AF
Muscle	0.20(4)		0.49(75)	0.14(3)	0.089(30)	GST
	0.068(10)	0.12(1)	0.20(3)	0.17(4)	0.059(11)	AF
Skin	0.39(17)		0.42(6)	0.41(9)	0.27(4)	GST
	0.097(13)	0.17(3)	0.28(2)	0.27(3)	0.18(1)	AF

[a] Figures in parentheses are the standard deviation of the last significant figure.

mixture was filtered from excess of the gold cyanide, and evaporated to dryness. The pale yellow solid was washed repeatedly with hot ethyl acetate and dried (5.0 mg, 10 μCi; 0.5% overall). The purity was checked by t.l.c. as described above. For use, the ^{35}S-material was diluted with inactive gold sodium thiomalate (1.5 mg), dissolved in water (3.5 cm^3), and adjusted to pH 5.1 by addition of sodium hydroxide solution.

[^{35}S]AF.—[^{35}S]-(2,3,4,6-Tetra-*O*-acetylglucopyranosyl)-thiopseudourea hydrobromide was synthesized as described by Hill and Sutton[23] from [^{35}S]thiourea and 2,3,4,6-tetra-*O*-acetylglucopyranosyl bromide (**B**). The labelled compound (44.9 mg, 92.2 μmol) was dissolved in water (3 cm^3) and added to a cold solution of potassium carbonate (12.3 mg, 92.2 μmol) in water (2 cm^3). To this was immediately added a solution of triethylphosphinegold acetate (92.2 μmol) in ethanol (2 cm^3), prepared as described above. The mixture was stirred and cooled for 1 h, reduced to half its volume, and cooled in ice. [^{35}S]AF crystallized and was collected (38.8 mg, 62%). Its purity was checked by t.l.c. as described above.

Administration, Sampling, and Assay.—Wistar rats of *ca.* 200 g body weight were used.

GST (20 mg, 51 μmol, *ca.* 500 μCi) was dissolved in water (20 cm^3) and millipore-filtered. Twenty rats were given injections (0.5 cm^3) in the right thigh muscle, and groups of five (^{199}Au) or three (^{35}S) were sacrificed at 2, 24, 48, and 168 h.

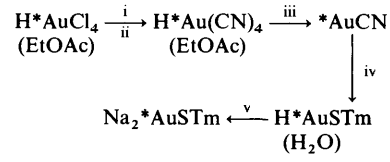
AF (25 mg, 40 μmol, *ca.* 1.00 mCi) was dissolved in ethanol (2.0 cm^3) and diluted to 25 cm^3 with water, to give a clear, colourless solution. Twenty rats were given oral doses of 0.50 cm^3, and groups of four were sacrificed after 2, 5, 24, 48, and 168 h.

In all cases, the major organs (kidney, liver, spleen, lung) and a bone joint were excised, and samples of skin, bone and muscle (left thigh) were taken. Blood samples were taken from the heart immediately after death, and stored in heparinized tubes. Samples were weighed and their gamma-activity measured and compared with those of standards prepared by dilution of 0.5 cm^3 of the original solution to 1.0 dm^3. For the ^{35}S work, tissue samples (200 mg, or less) were minced, kept at 55 °C overnight in Protosol solution (2 cm^3), treated with hydrogen peroxide (30%, 100 μl), and heated again to 55 °C for 30 min. After the samples had been cooled, Econofluor (10 cm^3) was added. Whole blood or red blood cells were similarly treated, using Protosol–ethanol mixtures. Bone and joint samples were solubilized by being heated with perchloric acid (60%; 0.2 cm^3) and hydrogen peroxide (30%; 0.4 cm^3) in a sealed vial, and heated at 60 °C until a clear solution was obtained. [**CAUTION**: SAMPLES MUST BE LESS THAN 500 mg, TO AVOID RISK OF EXPLOSION] After the samples had been cooled, Biofluor (10 cm^3) was added. The solutions were allowed to stand for 60 min in the counter before counting commenced. A known amount of dioctyl [^{35}S]sulphide standard was added, and the sample was counted again.

Activities per gram of organ were corrected for radioactive decay, and are quoted as a percentage of the total dose administered. The data are given in Tables 3 and 4.

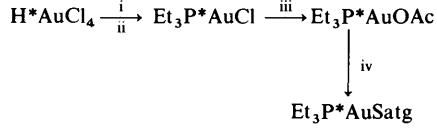
Results

Radiolabelling.—Simple methods have been devised and implemented for the synthesis of ^{199}Au-labelled GST and AF. The isotope is efficiently extracted by ethyl acetate from an *aqua regia* solution of the neutron-irradiated platinum target. Preparation of GST is easily effected *via* gold(I) cyanide (Scheme 1). No glycerol was added in this preparation, and



Scheme 1. Synthesis of [^{199}Au]Myocrisin (H_3STm = thiomalic acid). *Reagents:* i, aq. NaCN; ii, aq. HCl; iii, 100 °C; iv, aq. H_3STm; v, aq. NaOH

excess of thiomalic acid was removed by extraction with ethyl acetate before final neutralization. [^{199}Au]AF was prepared by a method analogous to that reported by Hill and Sutton.[23] However, the overall yield was significantly improved (to >70%) by *in situ* conversion of triethylphosphinegold chloride into the corresponding acetate (Scheme 2).



Scheme 2. Synthesis of [^{199}Au]Auranofin [tdg = thiodiglycol, $(HOCH_2CH_2)_2S$; HSatg = tetra-acetylthioglucose]. *Reagents:* i, tdg; ii, Et_3P; iii, AgOAc; iv, HSatg

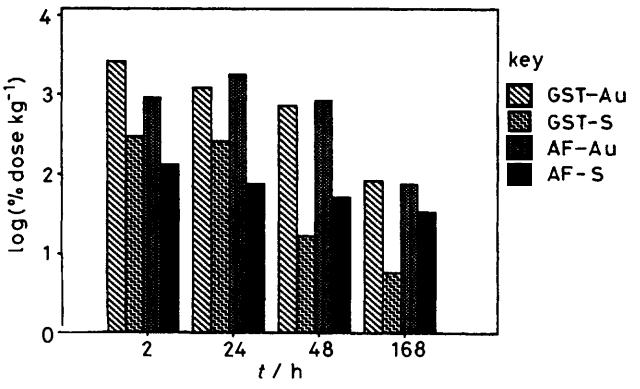


Figure. Gold and sulphur levels in whole rat blood at various times

^{199}Au *Distribution.*—The data for GST are broadly in agreement with one of the previous reports (Tables 1 and 4). For AF, the trends are similar but the levels observed here are considerably greater; this difference is presumably due to the different mode of administration, and is discussed further below.

In the first few hours, the percentages of gold from GST (GST-gold)* are considerably greater than those from AF (AF-gold)* in all organs (see Table 4). This is consistent with the known differences in rates of absorption of the two drugs, injected GST being taken up almost immediately, and orally administered AF much more slowly.[18,25] This is most apparent in the blood profiles (Figure); since the drugs must enter the blood stream first, the time distributions in other organs of necessity follow similar patterns. Beyond 24 h, there are no gross differences in gold distribution produced by the two drugs, although the data do appear to substantiate the claim[6,9] that AF leads to lower accumulation in liver and kidney.

GST-gold is present in the blood at high concentration at the first time-point (2 h; *ca.* 38% of the total dose). Clearance is rapid, and follows the biphasic pattern observed by Walz *et al.*[18,25] However, while the initial half-life observed here (17.1 ± 2.1 h) is similar to that reported earlier (17.3 h), the terminal values are significantly different (38.2 ± 2.7 *vs.* 62.4 h). This difference is presumably due to the lack of points between 48 and 168 h in the present work. For AF, the rate of absorption is much slower than for GST, but still exceeds that of removal during the first 24 h. At the end of 24 h, *ca.* 25% of the total administered gold was present in the blood stream. In this case, only a terminal clearance half-time can be calculated, 34.0 ± 1.0 h, which is very similar to that for GST-gold and to that previously reported (30 h).[18,25]

Accumulation of gold by kidney, liver, and spleen follow broadly similar patterns for the two drugs, with the levels of AF-gold being somewhat lower especially in the spleen. After 48 h, levels in these organs are effectively constant except for the kidney, which shows a slow increase for GST-gold.

For both drugs, the joint accumulates significantly more gold than the surrounding bone, indicating a concentration in the synovium. This level is effectively the same for both drugs, and falls only slowly, suggesting considerable long-term retention, as seen in both animal and patient studies.[19,26] Unfortunately, we were not able to monitor the bone marrow separately. The skin accumulates levels of gold comparable to the joints, which also decrease only slowly.

When the total mass of each organ is taken into account, retention beyond 48 h is principally in the kidney and liver, but a significant quantity is also present in the skin (Table 5). If these values are summed, about 50% of the total gold administered is present in these organs after 24 h. The subsequent decline is mainly due to clearance from the blood which is not compensated by organ-uptake; presumably this gold is largely eliminated. The lower relative total retention of AF-gold than GST-gold is barely significant statistically, but the trend is due to a drop (3%) in the liver content between 24 and 48 h, and to the constancy of kidney level between 24 and 168 h while the GST-kidney-gold level rises (6%).

^{35}S *Distribution.*—The present data for ^{35}S-GST agree reasonably well with only one of the previous reports involving ^{14}C labels (Tables 2, 5). The sulphur levels in all organs at all time points are considerably less than those of gold (Tables 1, 2, 4, 5, 7). Since the drug is quickly and completely taken into the blood stream, this can only indicate a rapid separation of the gold and the thiolate which is followed by much more efficient clearance of the thiolate. The highest levels are found in the kidney, which is consistent with observed high levels of urinary excretion..[17] Within the blood stream the sulphur label, like gold,[25,27] is predominantly present in the plasma.

The pattern for AF is somewhat different. At 2 h, the blood contains a higher proportion of the gold- than the sulphur-label, as for GST, but the other organs monitored show approximately equal amounts of the two labels (Table 7). The blood-sulphur levels are effectively unchanged between 2 and 5 h, and decrease only after this point (Table 5). These data confirm the previous observation that sulphur levels peak much earlier than gold levels and have a much shorter half-life.[15,21] Thus, although absorption of sulphur, like that of gold, is initially slow, clearance is both efficient and rapid. However, the sulphur label is uniformly distributed between cells and plasma, as gold is reported to be.[18,25] The higher relative proportion of AF-sulphur in the organs presumably reflects its more rapid absorption than gold. These data also imply considerable separation of the gold- and sulphur-containing moieties. The residual sulphur levels (at 168 h) are rather lower than for GST-sulphur in all organs except the joint.

Discussion

The data for the distribution of gold are broadly similar to those reported previously. However, it is evident that both the rate of absorption of AF-gold and the total amount absorbed are significantly greater than observed by previous workers, the difference being a factor of 4—5 (*cf.* Tables 1 and 4). When AF is orally administered as a solid, only 18—25% of the dose is absorbed, the remainder being rapidly eliminated, mainly fecally.[18,19] In the present study, effectively all of the AF-gold was absorbed. Thus, administration of a solution of the drug (in 5% aqueous ethanol) gives an enormous increase in the efficiency of absorption. The reason for this is not understood, but it implies that the AF may stay in solution in the stomach or gut or, possibly, be precipitated in very finely divided form, which makes it more reactive and/or resistant to elimination. Interestingly, however, despite the increase in extent of uptake, the kinetics of the AF-gold are effectively unchanged.

Clearance of AF-gold is significantly slower than that of GST-gold during the early stages, and the gold blood-levels are higher for AF at both 24 and 48 h. This must be indicative of a difference in the chemistry of the gold in the blood-stream between these two types of administered gold which is also reflected in the different distribution between serum and cells (see below).

The retention of gold by the kidney, liver, and spleen is

* This terminology is necessary because only the gold is being monitored; no information is available, from these data, about its molecular form, nor about the fate of the remainder of the drug molecule.

Table 5. Distribution of GST-sulphur and AF-sulphur (as ^{35}S, percentage of initial dose per gram) at various times after administration[a]

Organ	2 h	5 h	24 h	48 h	168 h	Drug
Blood cells	0.025(70)		0.014(2)	0.004(1)	0.0015(2)	GST
	0.143(38)	0.154(9)	0.072(13)	0.059(12)		AF
Blood serum	0.630(60)		0.550(30)	0.373(50)	0.0115(4)	GST
	0.128(46)	0.151(50)	0.078(13)	0.046(11)	0.008(4)	AF
Kidney	0.786(150)		0.306(40)	0.240(10)	0.086(10)	GST
	1.51(32)	0.91(31)	0.095(27)	0.048(10)	0.023(8)	AF
Liver	0.166(40)		0.080(8)	0.066(9)	0.043(7)	GST
	0.280(22)	0.141(13)	0.045(10)	0.022(3)	0.010(9)	AF
Spleen	0.076(30)		0.043(10)	0.040(10)	0.027(1)	GST
	0.173(27)	0.098(28)	0.034(6)	0.020(6)	0.014(2)	AF
Lung	0.153(20)		0.083(10)	0.060(10)	0.052(10)	GST
	0.235(23)	0.134(27)	0.041(8)	0.021(7)	0.025(3)	AF
Joint	0.013(3)		0.036(9)	0.036(10)	0.0044(3)	GST
	0.126(17)	0.102(24)	0.099(19)	0.078(10)	0.024(6)	AF
Bone	0.035(10)		0.033(9)	0.020(10)	0.009(1)	GST
	0.066(25)	0.061(13)	0.033(6)	0.025(7)	0.006(4)	AF
Skin	0.033(5)		0.042(8)	0.045(5)	0.025(60)	GST
	0.119(9)	0.117(38)	0.050(12)	0.050(1)	0.025(6)	AF
Muscle	0.035(8)		0.029(10)	0.031(2)	0.017(2)	GST
	0.089(13)	0.080(14)	0.018(1)	0.014(1)	0.010(3)	AF

[a] Figures in parentheses are the standard deviation of the last significant figure.

Table 6. Total gold content of organs (percentage of dose per whole organ) at various times[a]

	2 h		24 h		48 h		168 h	
Organ	GST	AF	GST	AF	GST	AF	GST	AF
Blood	38	13	16	25	10	12	1	1
Kidney	10	2	14	11	15	12	20	11
Liver	8	3	8	7	7	3	6	3
Skin	8	2	9	6	8	5	5	4
Sum	64	20	47	49	40	32	32	19

[a] The following organ masses were taken (per 200 g rat): blood, 14 g; kidney, 1.7 g; liver, 10 g; skin, 20 g.

Table 7. Gold:sulphur mole ratios at various times

	GST		AF		
Organ/time	2 h	24 h	2 h	5 h	24 h
Blood	8.2	4.3	6.5	8.5	21
Kidney	8.1	27	0.73	3.4	72
Liver	5.0	9.8	1.2	4.5	14
Spleen	10.5	17	1.6	5.9	29
Bone joint	31	9.7	0.95	2.4	4.5
Bone	6.9	6.7	1.7	2.5	9.4
Muscle	5.7	17	0.76	1.5	11
Skin	11.8	10	0.82	1.5	5.6

significantly less for AF than GST (Table 6): after 24 h these organs contain *ca.* 25% of the total gold administered as GST, but only *ca.* 16% of that given as AF (this difference is greater than the statistical uncertainties, which are *ca.* ±2%). In view of the possibility of toxic accumulation, this observation could be highly significant. Previous studies have also suggested that gold-retention may be less for AF,[18,19] but this could have been due simply to the low efficiency of uptake of the drug into the body. The present data suggest that there may be a fundamental difference between the drugs either in the absorption of gold from the blood or in its tightness of binding to the clearance organs. This again indicates possible differences in the blood-chemistry. On the other hand, the low levels of sulphur indicate that it and the gold follow rather different metabolic pathways. In particular, the sulphur-containing metabolites are efficiently cleared, principally *via* the kidney. These data would seem to suggest that separation of gold from the thiolate occurs at an early stage, *i.e.* in the blood stream or, for AF, perhaps in the intestine. Small residual levels of sulphur are presumably due to binding to endogenous thiols as disulphides, as shown by Jellum.[12,13] The lower residual levels of AF-sulphur suggest that thioglucose is less susceptible to disulphide formation, as might be expected.

An everted-gut experiment (hamster, rat) showed that unmetabolized AF does not pass through the intestinal wall.[28] Gold which had passed through the wall was shown to be present as the de-acetylated form of the drug, Et_3PAuSR' [HSR′ = 1-thio-β-D-glucopyranose]. Whether this is the actual material transmitted or whether it is formed by separate passage of the thiol and an Et_3PAu-moiety, which subsequently recombine, was not established, but it was shown that AF could be reformed by the addition of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose to the metabolite mixture. The fact that gold and sulphur show different rates of appearance in the blood stream suggests that gold–sulphur bond cleavage occurs either during absorption or in the blood stream (exchange reactions with other thiols are known to be rapid[29,30]), and that the released thiolate is more rapidly absorbed (and eliminated) than the gold-containing metabolite. The relatively slow, yet eventually complete, uptake of gold indicates that AF or its gold-containing metabolites are held in the stomach or intestine, and are not eliminated.

Elegant *in vitro* experiments by Shaw's group[31,32] have shown that both GST and AF (and, presumably therefore, the metabolites of the latter) react rapidly and completely with albumin. Binding occurs at the sulphydryl group of Cys-34. At high ratios of albumin to GST, as found in the blood-stream, the complex AlbSAuSTm (STm = disodium thiomalate) is formed. In *in vivo* processes, the thiomalate ligand would probably be displaced rapidly from this complex by reaction with endogenous thiols (*e.g.* glutathione). *in vitro*, gold from AF or Et_3PAuCl binds to albumin initially as $AlbSAuPEt_3$. If this last species were present in the blood, it might account for the lower initial rate of scavenging of AF-gold, and for the fact that the gold passes into the red cells. However, it has also been observed that a ^{32}P label shows very similar kinetic behaviour to ^{35}S, giving a maximum blood-level after only 1.5 h, and a clearance

half-life of 6—10 h.[18] This would suggest that considerable gold–phosphorus bond cleavage also occurs during or immediately after absorption. On the other hand, the different distribution of gold between cells and serum observed for the two drugs[18,25,27] clearly indicates that at least a proportion of the gold is in different chemical forms in the blood-stream in the two cases. It is also noticeable that AF-sulphur levels in the blood, although much lower than the gold levels, show similar partition between cells and serum; this might be taken to imply that the gold and the thiolate remain together long enough for cell-absorption to occur. Further clarification is needed of this important point. In the long-term, gold from either drug is presumably present in the serum as AlbSAuSR″ (HSR″ = endogenous thiol), which is consistent with the observation that both the cell–serum distributions and the clearance rates from the two drugs slowly become more similar (after a single dose).

In the kidney, gold is almost certainly bound, *inter alia*, as complexes with metallothionein. Gold from GST is known to displace zinc and cadmium from metallothionein.[33–35] AF does not react *in vitro*, but can plainly do so after having been metabolized. Any remaining gold-bound triethylphosphine is presumably released during this process. The phosphine is eliminated almost entirely *via* the urine, as its oxide.[18]

Both drugs give small but significant gold levels in the joint, which are 2—3 times higher than those in the surrounding bone. It has been suggested[26] that affected joints take up more gold than normal joints, but we have been unable to test this. Since the synovium is the affected tissue in rheumatoid arthritis, it is significant that the therapeutic metal reaches this area and that, once there, it is retained.

Conclusion

The rapid and effectively complete elimination of the sulphur label suggests that the thiolate parts of the drugs are not responsible for the therapeutic action. Since gold compounds other than thiolates are known to be effective, it seems likely that it is indeed the gold which is active. It appears that separation of the gold and thiolate moieties occurs early in the metabolic processes, probably in the blood stream, and that these two components of the drug are effectively independent thereafter. However, there are significant differences in gold distribution between GST and AF, which indicate that at least some of the administered gold retains the original ligands (or their metabolites) for a period long enough to affect their biochemistry.

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