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Triethylphosphine gold: cellular uptake and disposition after single and repeated oral doses in rats

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The tissue and subcellular pharmacokinetics of gold following single and repeated oral doses of triethylphospine gold (auranofin) has been studied in rats. After a single dose, the tissue and subcellular gold levels were 5–10 times lower than those reached with injectable gold compounds. In the liver tissues, gold concentrations peaked within 24 h followed by a biphasic clearance, with an initial rapid phase ($t_{\frac{1}{2}}$ 32 h) and a slow terminal phase ($t_{\frac{1}{2}}$ 11 days). Renal gold concentrations continued to increase for 3 to 5 days and then decreased exponentially with a first order $t_{\frac{1}{2}}$ of about 7 days. Intracellularly, between 60–80% of hepatic and 50–70% of renal gold was present in the cytosol. In rats given repeated doses of auranofin, the hepatic and renal gold concentrations were 3–5 times higher than those measured after a single dose. The proportion of cellular gold present in the cytosol was markedly lower, with 43% in the liver and 30% in kidney tissues. In both the liver and kidney, gold concentrations were dose-dependent, whereas in the gastrointestinal tissues the increases as a function of dose were minimal.

The efficacy of gold compounds in the treatment of rheumatoid arthritis is well established (Empire Rheumatism Council 1961). However, conventionally used gold compounds are poorly absorbed orally, and therefore are administered intramuscularly at weekly intervals. The injected gold is rapidly absorbed into the systemic circulation and transported to the tissues, resulting in high concentrations of the metal, particularly in the tissues of the reticuloendothelial system (Vernon-Roberts et al 1976; Sharma & McQueen 1979). The tissue uptake of gold is cumulative and in the long-term may result in unpredictable adverse effects such as bone-marrow suppression, dermatitis, diffuse pulmonary injury and nephrosis (Kay 1976; Gould et al 1977; Gibbons 1979).

Recently an orally administered gold complex, triethylphosphine gold (auranofin), has been developed for clinical use in the treatment of rheumatoid arthritis. It is clear that there are significant differences in serum or plasma pharmacokinetics and tissue uptake of gold, between the orally administered gold complex and the intramuscularly injected forms (Walz et al 1980). While some studies with parenteral gold show a correlation between plasma or serum gold concentrations and clinical response or toxicity (Krusius et al 1970; Lorber et al 1975), most studies have not (Gerber et al 1972; Gottlieb & Smith 1974). More recent studies suggest that the effector sites for clinical response (Mirabelli & Crooke 1983; Sharma 1983) and toxicity (Eiseman & Alvares 1978; Nechay 1980; Sharma & McQueen 1982) may both be at intracellular gold-binding sites. In this context the time course and interrelationships of gold concentrations in various tissues and their subcellular compartments may be critical. We have therefore investigated, in rats, the tissue and subcellular pharmacokinetics of gold following single and repeated oral doses of triethylphosphine gold.

MATERIALS AND METHODS

Single dose kinetics

Male Wistar rats (≈140 g) were orally administered Au 5 mg kg^{-1} as auranofin (triethylphosphine gold suspended in 0·5% tragacanth solution). The animals were killed in groups of three at 1, 2, 4, 7, 12, 17, 24 h and at 2, 5, 10, 15, 20, 25 and 30 days following the auranofin dose. The liver and kidney tissues corresponding to each sampling time were pooled, homogenized, and the various subcellular organelles isolated and purified as described below. Gold concentrations were determined in the homogenates and sub-cellular fractions and are expressed as Au μg g^{-1} tissue or subcellular fraction in the kidney and as ng g^{-1} in the liver. The experiment was repeated and the concentration values plotted are an average of the duplicate results.

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Multiple dose study

In this experiment the rats were dosed orally in groups of three with Au 5, 10 or 15 mg kg^{-1} per day as auranofin for three days. After the last dose the animals were fasted for 24 h and then killed. The liver, kidney and gastrointestinal tissues (small intestine) were processed as for the single dose study. The experiment was repeated to obtain results in triplicates (i.e. 9 animals per point).

Subcellular fractionation

The pooled tissue samples (liver, kidney and small intestine) from each group above, were minced in ice-cold 0·25 M sucrose solution and then rinsed twice to minimize contamination from extracellular gold.

The tissues were then homogenized in 0·25 M sucrose at 4 °C. The homogenates were initially subjected to differential centrifugation and the crude nuclear (N), mitochondrial (M), lysosomal (L), and microsomal (P) pellets obtained were then purified as described by Sharma & Edwards (1983). All steps in subcellular fractionations were at 4 °C using a Beckman L-5 ultracentrifuge and a Sorvall RC-2B refrigerated centrifuge. The supernatants obtained at the end of the differential centrifugations were retained as the cytosol (C).

Gold analysis

Sub-samples of various tissue homogenates and the corresponding subcellular fractions were digested in concentrated nitric acid at 75 °C for at least 4 h. The digests were appropriately diluted with 10 mM nitric acid solution and analysed for gold by flameless atomic absorption spectroscopy as described elsewhere (Sharma 1982). A deuterium continuum source was used for background correction.

RESULTS

Tissue pharmacokinetics

The uptake and decay of gold in the liver and kidney tissues following a single oral dose of auranofin is shown in Fig. 1. In the liver, gold concentrations increased rapidly reaching maximum levels within 24 h. Thereafter the tissue clearance of gold was bi-exponential with an initial 1st order elimination half-life ($t_{\frac{1}{2}}$) of about 32 h and a considerably slower terminal $t_{\frac{1}{2}}$ of about 11 days. In the kidney tissues, gold concentrations continued to increase for about 3–5 days and then decreased exponentially with a 1st order $t_{\frac{1}{2}}$ of 7·2 days. The gold levels in the kidneys were about 10 times higher than those in the liver. Relative to the peak gold values in the liver (0·54 μg g^{-1}) and kidney (4·4 μg g^{-1}), about 90 and

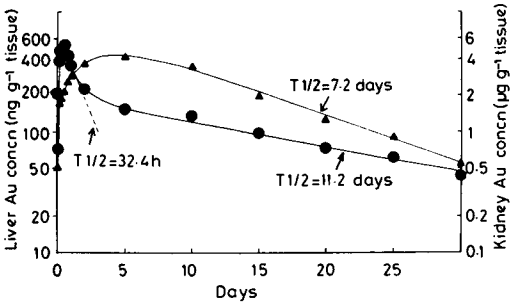

FIG. 1. Time-dependent uptake and clearance of gold in the liver (●) and kidney (▲) tissues, following a single oral dose of auranofin.

85% respectively of the tissue gold was eliminated during the 30 days after a single dose of Auranofin.

Subcellular distribution and clearance

The time-course of gold in subcellular compartments of the liver tissues is shown in Fig. 2. Following a single dose of auranofin, gold levels peaked within 48 h in all fractions (nuclear, mitochondrial, etc), except the lysosomal, which showed a continued increase for up to 5 days. (The tissue and cytosolic gold concentrations are expressed as μg g^{-1} tissue, whereas those in the subcellular particulate fractions (N, M, L and P) are as μg g^{-1} subcellular fraction.) The uptake and clearance of gold in the kidney (Fig. 3) was similar to that in liver, except that the cytosolic gold levels continued to increase and peaked on the 5th day after the auranofin dose. In both the liver and the kidneys, concentrations of intracellular gold were markedly higher in the lysosomes, microsomes and cytosol compared with those in the nuclear and mitochondrial fractions. Detectable amounts of gold were present in all subcellular compartments even after 30 days.

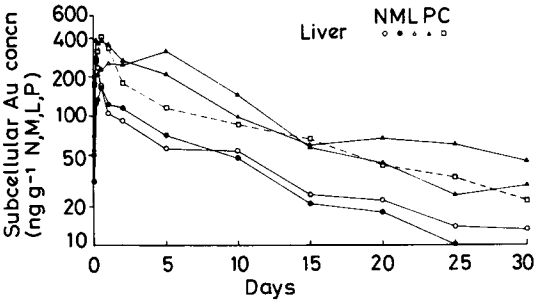

FIG. 2. Subcellular distribution and clearance of gold in the liver after a single oral dose of auranofin. ○ Nuclear, ● mitochondrial, △ lysosomal, ▲ microsomal, □ cytosol.

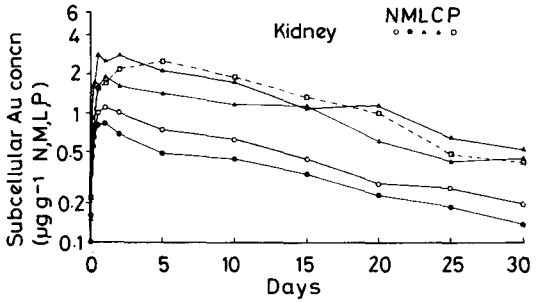

FIG. 3. Subcellular distribution and clearance of gold in the renal tissues after a single oral dose of auranofin. Symbols as in Fig. 2.

Repeated dose study

In this part of the study, the rats were given Au 5, 10 or 15 mg kg^{-1} per day as auranofin for three days (i.e. a total of three doses at each dose level). In the gastrointestinal tissues (small intestine) the increases in tissue and subcellular concentrations of gold (Table 1) were relatively small in comparison with the large increases in the auranofin dose from Au 5 to 15 mg kg^{-1}. At 24 h after the third dose, the highest gold concentrations measured were in the microsomes. On an average, about 41% of the cellular gold was present in the cytosol.

The concentrations of gold in the hepatic and renal tissues (Tables 2 and 3) showed markedly larger increases with the increase in auranofin dose than those in the gastrointestinal tissues. Intracellularly, however, these increases were mainly reflected in the cytosol. It was also evident that in the liver mitochondrial fractions (Table 2), gold concentrations were higher (Student's t-test, $P < 0{\cdot}05$) than in the nuclei at all three dose levels. However, in the corresponding renal fractions (Table 3), the nuclei consistently contained significantly ($P < 0{\cdot}05$) higher gold concentrations than those in the mitochondria.

After a single dose (Au 5 mg kg^{-1}) of auranofin (Fig. 1), the peak liver and kidney tissue gold concentrations were 0·54 and 4·4 μg g^{-1} respectively. The concentrations reached following similar (5 mg kg^{-1}) repeated doses (Tables 2, 3) were about 2 to 3 times higher, resulting in correspondingly higher gold concentrations in the subcellular organelles. But the concentrations in the cytosol were only marginally higher in multiple dosed rats compared with the single dosed.

Table 1. Gold concentrations in the gut tissues and subcellular compartments of rats following repeated oral doses of auranofin. The gold is in Au μg g^{-1} tissue or fraction.

Sample	Auranofin dose, mg kg^{-1} per day 5	10	15
Gut tissue	0·75	0·91	1·18
Subcellular fractions			
Nuclear (N)	0·34	0·39	0·52
Mitochondrial (M)	0·33	0·46	0·56
Lysosomal (L)	0·40	0·57	0·60
Microsomal (M)	1·18	0·88	1·31
Cytosol‡	0·33	0·38	0·44

‡ The gold concentrations in the cytosol are expressed as Au μg g^{-1} tissue. The values for gold concentrations are an average of 6 separate analyses (3 experiments with duplicate analysis) with an average coefficient of variance of ±14%.

Table 2. Hepatic tissue and subcellular gold concentrations following repeated oral doses of auranofin (Au μg g^{-1} tissue or fraction).

Sample	Auranofin dose, mg kg^{-1} per day 5	10	15
Liver tissue	1·78	2·89	4·49
Subcellular fractions			
Nuclear	1·04	0·90	0 95
Mitochondrial	1·21	1·46	1·52
Lysosomal	1·52	1·71	2·06
Microsomal	1·89	1·45	1·11
Cytosol‡	0·76	1·14	1·96

‡ The gold concentrations in the cytosol are expressed as Au μg g^{-1} tissue. The values for gold concentrations are averages as in Table 1: coefficient of variance ±11%.

DISCUSSION

Previous metabolic studies of antiarthritic gold compounds have been concerned primarily with pharmacokinetics in blood (Pedersen & Graabaek 1980; Walz et al 1980), synovial fluid (Gerber et al 1972) and with mechanisms of action (Goldberg et al 1981; Lipsky & Ziff 1982; Sharma 1983). Although several studies have also reported concentrations of

Table 3. Renal tissue and subcellular gold concentrations following repeated oral doses of auranofin (μg Au g^{-1} tissue or fraction).

Sample	Auranofin dose, mg kg^{-1} per day 5	10	15
Kidney tissue	9·04	13·54	16·55
Subcellular fractions			
Nuclear	2·09	2·33	2·46
Mitochondrial	1·28	1·43	1·93
Lysosomal	2·59	2·52	2·63
Microsomal	3·90	2·28	1·77
Cytosol‡	2·93	4·85	6·06

‡ The gold concentration in the cytosol are expressed as Au μg g^{-1} tissue. The values for gold concentrations are averages as in Table 1: coefficient of variance ±11%.

gold in various tissues (Gottlieb et al 1972; Kamel et al 1978) and subcellular organelles (Penneys et al 1976; Lawson et al 1977), few authors have investigated the time/dose-dependent cellular uptake and distribution of the metal after administration of clinically used gold compounds (Sharma & McQueen 1980; Mogilnicka & Webb 1983). The present study quantitates the cellular and subcellular concentrations of gold in rat liver, kidney and gastrointestinal tissues as a function of time and dose.

It is clear from the results that following oral administration of auranofin, the tissue and subcellular gold concentrations were markedly lower than those reached with injectable gold compounds (Kamel et al 1978; Sharma 1983). For instance, rats injected with Au 2·5 mg kg^{-1} as gold sodium thiomalate reach peak kidney and liver gold concentrations of about 43 μg g^{-1} and 3 μg g^{-1} tissue respectively at 24 h after a single dose (Sharma 1983). In comparison the maximum concentrations reached with an oral dose of Au 5 mg kg^{-1} as auranofin were approximately ten-fold lower, with 4·4 μg g^{-1} in the kidney and 0·54 μg g^{-1} in liver tissues. In auranofin, gold is bound to a substituted glucose molecule via a sulphur atom and has organophosphate radicals as additional binding. Thereby the complex becomes amphiphile and is absorbed partially (20–30%) from the gastrointestinal tract (Blocka & Landaw 1983). The markedly lower tissue gold concentrations measured following oral administration of auranofin, may therefore be largely due to a low absorption of the compound from the gastrointestinal tract.

With the parenteral gold compounds, tissue uptake of gold is rapid and the clearance from both the liver and kidneys is biphasic with an initial rapid phase ($t_{\frac{1}{2}}$ about 6 days) followed by a slow terminal phase (Sharma & McQueen 1980). Thus with repeated injections (Sharma 1983) tissue gold concentrations increase rapidly. After a single oral dose of auranofin the kinetics of gold in the rat liver was similar to that reported for parenteral gold. However, in the kidney, clearance of the metal continued at an approximately constant rate through to the 30th day after the auranofin dose, with a $t_{\frac{1}{2}}$ about 7 days (Fig. 1). It is possible that this difference may partly be due to the longer half-life and cellular retention of auranofin gold in the blood (Walz et al 1980; Herrlinger et al 1982), thus resulting in a more gradual uptake into the renal tissues and subsequent clearance, compared with parenteral gold.

Recent studies show that biochemical activities of some physiologically important enzymes, such as δ-aminolevulinic acid dehydratase and NADPH-cytochrome C reductase (Eiseman & Alvares 1978), adenosine triphosphatase (Nechay 1980), and mitochondrial L-malate dehydrogenase (Friedman et al 1982) are significantly inhibited by gold compounds, and are highly sensitive to gold concentrations particularly in the liver and kidney tissues. Hence the concentrations of gold in various subcellular compartments are likely to be critical with respect to the cellular toxicity of gold. In the present study, after a single dose of auranofin between 60–80% of hepatic and 50–70% of renal gold was present in the cytosol. This is in contrast to that reported for injectable gold compounds (Penneys et al 1976; Sharma & McQueen 1980), where up to 70% of the cellular gold was present in the organelles, in particular the lysosomal bodies. In the liver and kidney, an inducible protein, metallothionein (MT), plays a significant role in the sequestration and localization of cytosolic gold (Mogilnicka & Piotrowski 1979). However the extent of gold binding to MT is limited by the amount of pre-synthesized MT (e.g. synthesis induced by zinc) present intracellularly and the ability of gold itself to induce additional synthesis (Sharma & McQueen 1982). It is possible, therefore, that at low tissue gold concentrations a large proportion of the cellular gold is localized in the cytosol via additional incorporation into storage proteins such as MT (which may bind as much as 50% of the cytosolic gold). At much higher tissue gold levels (as encountered with injectable gold compounds), the binding to cytosolic ligands (including MT) may plateau, resulting in an increased uptake into the organelles, which may cause a greater susceptibility to adverse cellular effects of gold. Such a mechanism may to some extent also explain the clinical observations (Laurence 1976; McKenzie 1981) which demonstrate that patients receiving small doses of injectable gold compounds or oily suspensions of the drug, rather than the fast-absorbing aqueous solution, show a lower susceptibility to gold toxicity. Indeed, recent clinical studies of auranofin itself show an absence of serious penicillamine-like adverse effects normally associated with injectable gold (Bandilla et al 1982; Barraclough et al 1982; Palmer et al 1983).

In animals given repeated doses (Au 5 mg kg^{-1}) of auranofin (Tables 2, 3), the hepatic and renal gold concentrations were between 3 to 5 times higher than those measured (at 24 h) after a single dose (Fig. 1). Furthermore, the proportion of cellular gold present in the cytosol was markedly lower, with about 43% in the liver and 32% in kidney tissues. These results

also support the suggestion that at high tissue gold concentrations, the binding of gold to cytosolic ligands may plateau and the proportion associated with subcellular organelles subsequently increase.

In both the liver and kidney, gold concentrations were, as expected, dose-dependent (Tables 2 and 3). However in the gastrointestinal tissues (small intestine), the increases in gold concentrations as a function of dose were minimal (Table 1). Kamel et al (1978) have shown that gold concentrations in stomach tissue 5 h after oral administration of triethylphosphine gold was high compared with concentrations in other gastrointestinal tissues, but became much more comparable with them after 24 h. The reason(s) for these differences in the concentrations of gold in gastrointestinal tissues are not clear, although to some extent these may be attributable to the prevalance of diarrhoea at the higher doses of 10 and 15 mg kg^{-1}. It appears that although intestinal cells may act as a mucosal transport mechanism for gold, the cellular absorption of the metal in the intestine is not cumulative.

In chrysotherapy, the adverse effects of gold drugs are dose-dependent and cumulative. The subcellular distribution of gold generally reflects the extent of its interactions with organelles, membranes and the cytosolic ligands. With auranofin (triethylphosphine gold), the markedly lower cellular accumulation of gold, and the high localization (over 50% of cellular gold) in the cytosol, largely bound to proteins including metallothionein, may correspondingly reflect lower non-specific interactions with organelles and their biochemical processes of physiological importance. It is therefore suggested that a similar mechanism in man may provide a greater patient tolerance of the drug in the treatment of rheumatoid arthritis.

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