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EFFECTS OF THE CHRYSOTHERAPEUTIC AGENTS AURANOFIN AND GOLD SODIUM THIOMALATE ON HEPATIC AND RENAL DRUG METABOLISM AND HEME METABOLISM

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Abstract—These studies were designed to investigate the effects of the chrysotherapeutic agents auranofin and myochrysine (GST) on hepatic and renal drug-metabolizing enzymes and heme metabolism. Male Sprague–Dawley rats were either administered a single dose of auranofin (17, 34, or 68 mg/kg, p.o.) or administered daily doses of auranofin (0.2, 0.6, 2, 9, or 40 mg/kg/day, p.o.) or GST (1.2 or 5.8 mg/kg/day, i.p.) for 3 or 14 days. Rats were killed 24 hr after the final treatment, and subcellular fractions of liver and kidney were prepared. Cytochrome P-450 (P-450) content and ethoxycoumarin-*O*-deethylase (ECOD), benzphetamine-*N*-demethylase (BPND), δ-aminolevulinic acid (ALA) synthetase, and heme oxygenase activities were determined. Twenty-four hours following single doses of auranofin, no effects on hepatic P-450, ECOD, or BPND were observed. Treatment with the positive control compounds, $CoCl_2$ (60 mg/kg) and Co-protophorphyrin IX (33 mg/kg), produced decreases in all three variables at 24 hr. Auranofin, at 2 mg/kg, and GST treatment, at both doses, reduced hepatic P-450 and ECOD activity at 3 days. This effect was reversed with continued treatment for 14 days. BPND activity was unaffected at 3 days but was decreased at 14 days. Heme oxygenase activity was enhanced at 3 days and had returned to control activity at 14 days, while ALA synthetase was unaffected. With the exception of heme oxygenase, which was increased, renal variables were unaltered at 3 days. At 14 days, renal P-450 content was decreased in the high-dose auranofin group, heme oxygenase activity was increased in all groups, and ALA synthetase activity was elevated in high-dose auranofin animals. These data indicate that, at doses twenty times the human dose, auranofin and GST administration produced reversible decreases in hepatic and renal P-450 which may be the result of altered heme metabolism.

Chrysotherapeutic agents have long been used in the treatment of rheumatoid arthritis and historically these compounds (gold sodium thiomalate; aurothioglucose) have been administered parenterally [1]. The gold from these compounds has been shown to accumulate in the liver and kidney of both humans [2] and rats [3]. Many of the toxic effects in rats caused by gold sodium thiomalate (Myochrysine) [4], in particular the nephropathy, are similar to those seen with other heavy metals [5, 6]. Gold sodium thiomalate (GST) also has been shown to reduce cytochrome P-450 content in liver and kidney following a single high dose (75 mg/kg) in rats [7] and after three doses (25 mg/kg) in pregnant rats [8]. It has been proposed that the GST effects are the result of gold-mediated increases in heme oxygenase activity leading to enhanced heme degradation and decreases in hepatic hemoproteins including P-450 [7, 8]. These alterations in heme metabolism are consistent with well-established effects of transition metals on heme catabolism [9, 10]. While single high-dose GST treatment causes changes in heme metabolism, repeated treatment (20 days) at lower doses does not alter hepatic heme metabolism [7]. The absence of effects with repeated GST treatment could be the result of the dose being below the effect threshold, the short treatment period, or that the decreases produced early in treatment were reversed by the 20-day time point [7, 8]. Reversal of these effects would be consistent with earlier reports that compensatory increases in δ-aminolevulinic acid (ALA) synthetase activity are seen in response to transition metal stimulation of heme oxygenase activity [11, 12].

Auranofin [(2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato-*S*)(triethylphosphine)gold; Ridaura; SKF D-39162] is a recently developed orally active chrysotherapeutic agent with effectiveness similar to that observed with GST [13]. Although the animal toxicity noted with auranofin is similar to that seen with GST [4], the accumulation of auranofin-associated gold in rat liver and kidney [3, 14] is markedly less than that observed following GST treatment [3]. Consequently, it is of interest to determine whether auranofin treatment affects hepatic and renal drug metabolism and heme anabolism and catabolism in a manner similar to GST. The studies described here were designed to ascertain to what extent similarities exist between the effects observed with a parenteral chrysotherapeutic agent (GST) and auranofin, a lipophilic orally active chrysotherapeutic agent.

METHODS

Animals. Male Sprague–Dawley rats (CD/CrlBR) were obtained from the Charles River Breeding Laboratories (Kingston, NY). Rats were

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supplied and maintained virus-antibody negative, as determined by screening of sentinel animals for RCV/SDA, PVM, and Sendai virus. Animals were housed in Bioclean rooms in stainless steel wire cages at a temperature of 72 ± 2°F and a relative humidity of 50 ± 10% with a 12-hr light–dark cycle (7:00 a.m. to 7:00 p.m.). Food (Purina 5002, Ralson-Purina, St. Louis, MO) and water were available *ad lib.* Rats were quarantined for 2 weeks prior to use. Rats weighing 200–275 g were used in these experiments and animals were randomly assigned to treatment groups (four rats/group).

Chemicals. Auranofin obtained from SK & F Laboratories (Philadelphia, PA) was used in these studies. Gold sodium thiomalate was purchased from the Aldrich Chemical Co. (Milwaukee, WI). Tragacanth was obtained from A. H. Thomas (Philadelphia, PA), and a 0.5% suspension prepared in ultrapure water was used as the suspending agent for auranofin. Cobalt-protoporphyrin IX was purchased from Porphyrin Products (Logan, UT). All other chemicals were obtained from commercial suppliers and were reagent grade or better.

Treatments. Rats were treated daily (8:00 to 10:00 a.m.) for 1, 3 or 14 days. Auranofin-dosing suspensions were prepared fresh daily in 0.5% tragacanth using a mortar and pestle. Dosing solutions of GST were prepared daily using ultrapure water. In the single-dose study, rats received 17, 34, or 68 mg/kg (p.o.) of auranofin. Co-protoporphyrin IX (33 mg/kg, s.c.) [15] and $CoCl_2$ (60 mg/kg, s.c.) [15] were used as positive controls in this study. In the multidose studies, auranofin was administered at 0.2, 0.6, 2.0, 9.0, or 40 mg/kg/day (p.o.) for 3 and 14 days. Gold sodium thiomalate was administered at 1.2 or 5.8 mg/kg/day (i.p.) for 3 and 14 days. The GST doses of 1.2 and 5.8 mg/kg were theoretically gold bioequivalent to the auranofin doses of 9 and 40 mg/kg respectively. This calculation was based on gold content of 29% for auranofin and 50% for GST and bioavailability of 25% for orally administered auranofin and 100% for parenterally administered GST.

Tissue collection and subcellular fractionation. Rats were anesthetized (methoxyflurane, Pitman-Moore, Washington Crossing, NJ) 24 hr following the final treatment, and blood samples were obtained by cardiac puncture. Liver and kidneys were rapidly removed, weighed, and washed in ice-cold 1.15% KCl. Kidneys were decapsulated and the renal pelvis removed; tissues were minced, and rewashed in ice-cold 20 mM Tris–HCl, pH 7.4, containing 1.15% KCl. Minced livers and kidneys were homogenized in 20 mM Tris–HCl, pH 7.4, containing 1.15% KCl using either a Potter–Elvehjem (glass–teflon) homogenizer (liver) or a polytron (kidney). The homogenates were centrifuged (10,000 *g* for 20 min at 4°), and the supernatant fraction was decanted and then centrifuged (100,000 *g* · hr, at 4°) to sediment the microsomes [16]. The 100,000 *g* supernatant (cytosol) was saved and frozen at −75°. The pellet (microsomes) was resuspended in 20 mM Tris–HCl, pH 7.4, containing 5.4 mM Na_2EDTA and 250 mM sucrose (approx. 15–25 mg protein/ml) and frozen at −75°.

Drug-metabolizing enzyme assays. Assays for hepatic microsomal activity of the following enzymes were performed: benzphetamine-*N*-demethylase (BPND) [17], ethoxycoumarin-*O*-deethylase (ECOD) [18], and aryl hydrocarbon hydroxylase (AHH) [19]. The activity of ECOD was also assayed in renal microsomes. Protein concentrations were measured using the Biuret method [20]. Hepatic microsomal cytochrome P-450 (P-450) content was determined from reduced CO difference spectra, using an extinction coefficient of 91 cm^{-1} mM^{-1} [21]. Renal microsomal P-450 concentrations were determined from CO saturated, dithionite reduced difference spectra using an extinction coefficient of 104 cm^{-1} mM^{-1} [22].

ALA synthetase and heme oxygenase assays. Mitochondrial ALA synthetase activity [23] was determined on fresh preparations using the 10,000 *g* pellet as the source of mitochondria. The pellets were resuspended in 2.5 vol. of 100 mM glycine buffer containing 100 mM sodium citrate, 50 mM sodium phosphate, pH 7.4, 50 mM Tris–HCl, pH 7.4, 167 mM sucrose, 20 mM $MgCl_2$, and 17 mM Na_2EDTA. The standard incubation mixture (320 μl) contained 10–20 mg of protein, 0.3 mM pyridoxal-5′-phosphate and 0.8 mM ATP. The mixture was incubated at 37° for 60 min. The incubations were terminated by the addition of 150 μl of 15% trichloroacetic acid and centrifuged at 4000 *g* for 30 min. A 300-μl aliquot of supernatant fraction was combined with 135 μl of 1 M sodium acetate and 15 μl of acetylacetone and incubated at 85° for 15 min. After cooling, the pH was adjusted to 7 using 110 μl of 0.5 M Na_2HPO_4 containing 1 N NaOH, and the mixture was extracted with methylene chloride (750 μl) and then centrifuged at 2500 *g* for 5 min. Modified Ehrlich's reagent [24] (400 μl) was added to a 400-μl aliquot of aqueous phase and permitted to stand for 15 min. The absorbancy was measured at 555 nm, and ALA concentration was calculated using an extinction coefficient of 58 mM^{-1} cm^{-1} [24].

Microsomal heme oxygenase activity [25] was determined kinetically. The standard incubation mixture (3 ml) contained at a final concentration the following: 100 mM $NaPO_4$, pH 7.4, 0.85 mM glucose-6-phosphate, 3 units/ml glucose-6-phosphate dehydrogenase, 2 mM $MgCl_2$, 17 μM hemin, and 250 μl of hepatic cytosol (10 mg of cytosolic protein) from untreated rats. Solutions were heated to 37°, 10–20 mg of microsomal protein was added, the mixture was transferred to a quartz cuvette, a stirring bar was inserted, and the cuvette was placed in a temperature-controlled holder. The absorbancy was monitored using an Aminco DW-2c spectrophotometer (SLM-Aminco, Urbana, IL) in the dual beam mode (466–530 nm) for 3 min to determine the baseline. The reaction was initiated by the addition of NADPH (180 μM final concn), and the change in absorbancy was monitored for 5–10 min. Bilirubin concentration was calculated using an extinction coefficient of 40 mM^{-1} cm^{-1} [26].

Statistical analysis. The data were analyzed using a completely randomized analysis of variance. Treatment means were compared with control means using the least significant difference (L.S.D.) test [27]. Dose–response trends were evaluated by linear regression analysis [27]. The 0.05 level of probability was used as the criterion of significance.

Table 1. Effects of single doses of auranofin, Co-protoporphyrin IX, and $CoCl_2$ on hepatic cytochrome P-450 enzyme activities

Treatment*	Cytochrome P-450 (nmoles/mg protein)	BPND [nmoles HOCH·(mg protein)$^{-1}$·min^{-1}]	ECOD •[nmoles 7-OH-coumarin·(mg protein)$^{-1}$·min^{-1}]
Control	0.627 ± 0.051	1.27 ± 0.13	2.17 ± 0.31
Auranofin (17 mg/kg)	0.595 ± 0.022	1.31 ± 0.23	2.07 ± 0.14
Auranofin (34 mg/kg)	0.823 ± 0.139	1.44 ± 0.24	2.15 ± 0.44
Auranofin (68 mg/kg)	0.644 ± 0.078	1.28 ± 0.05	2.03 ± 0.19
$CoCl_2$ (60 mg/kg)	0.508 ± 0.057	0.74 ± 0.04†	1.04 ± 0.10†
Co-protoporphyrin IX (33 mg/kg)	0.423 ± 0.045	0.66 ± 0.013†	0.54 ± 0.05†

Data are expressed as mean ± S.E. (N = 4).
* Rats were killed 24 hr following a single treatment.
† Significantly different from control at $P < 0.05$.

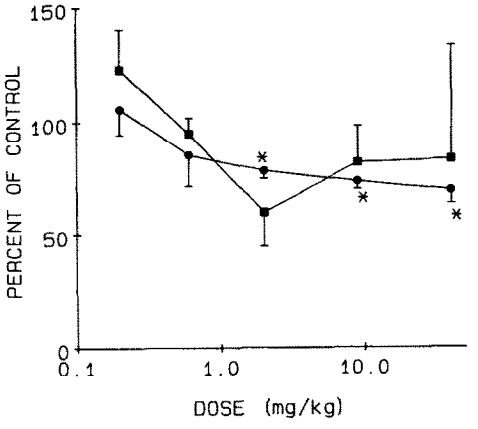

Fig. 1. Auranofin effects on hepatic cytochrome P-450. Cytochrome P-450 content was determined following 3 (●) or 14 (■) days of auranofin administration. Data are presented as the percent of the corresponding control values (3 days, 0.599 ± 0.009; 14 days, 0.592 ± 0.050 nmoles P-450/mg protein) with each point representing the mean ± S.E.M. (N = 4). Key: (*) significantly different from control at $P < 0.05$.

RESULTS

Single-dose studies. Reductions in body weight were noted 24 hr following a single auranofin treatment at all three doses. Administration of 68 mg/kg caused a decline (89% of control) in liver/body weight ratio, but no gross changes in hepatic integrity, as judged by serum alanine aminotransferase, were detected (data not shown). Single doses of auranofin did not alter hepatic P-450 content or BPND or ECOD activities (Table 1). In contrast to auranofin, Co-protoporphyrin IX and $CoCl_2$ markedly reduced BPND activity to 50–60% of control and ECOD activity to 25–50% of control 24 hr following a single dose.

Three-day studies. Administration of auranofin for

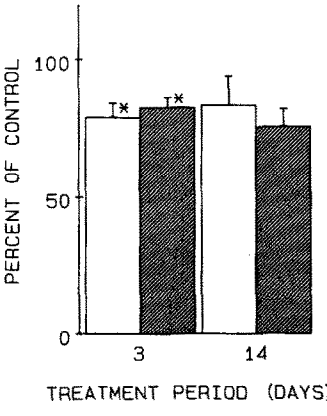

Fig. 2. GST effects on hepatic cytochrome P-450. Cytochrome P-450 content was determined following 3 or 14 days of GST administration at doses of 1.2 (□) and 5.8 (▨) mg/kg. Data are presented as the percent of the corresponding control values (3 days, 0.599 ± 0.009; 14 days, 0.592 ± 0.050 nmoles P-450/mg protein) with each point representing the mean ± S.E.M. (N = 4). Key: (*) significantly different from control at $P < 0.05$.

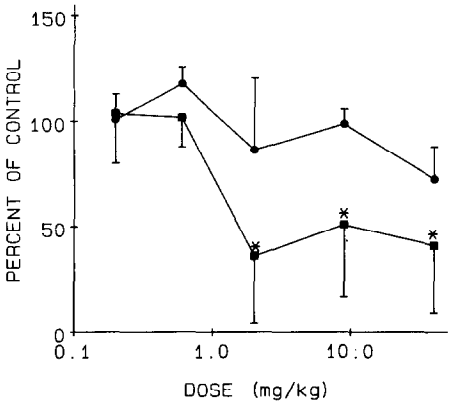

Fig. 3. Auranofin effects on hepatic BPND activity. BPND activity was determined following 3 (●) or 14 (■) days of auranofin administration. Data are presented as the percent of the corresponding control values (3 days, 0.98 ± 0.24; 14 days, 1.62 ± 0.19 nmoles HOCH/min/mg protein) with each point representing the mean ± S.E.M. (N = 4). Key: (*) significantly different from control at $P < 0.05$.

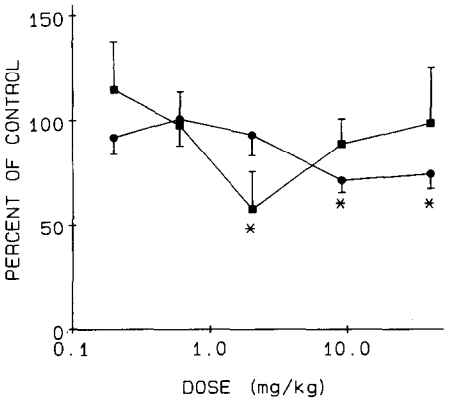

Fig. 5. Auranofin effects on hepatic ECOD activity. ECOD activity was determined following 3 (●) or 14 (■) days of auranofin administration. Data are presented as the percent of the corresponding control values (3 days, 1.22 ± 0.18; 14 days, 0.91 ± 0.07 nmoles 7-OH coumarin/min/mg protein) with each point representing the mean ± S.E.M. (N = 4). Key: (*) significantly different from control at $P < 0.05$.

3 days at 40 mg/kg/day slightly reduced the rate of body weight gain and liver to body weight ratios. At the lower doses of auranofin and GST, no significant effects on body weight gain and liver to body weight ratio were noted (data not shown). Three days of auranofin treatment produced dose-dependent decreases in hepatic P-450 content with a maximum decrease of 30% (Fig. 1). Administration of GST for 3 days also reduced hepatic P-450 values to a similar extent (Fig. 2). Hepatic BPND activity at 3 days was unaffected by auranofin (Fig. 3) or GST treatment (Fig. 4), whereas ECOD activity was depressed significantly (70% of control) by the mid and high doses of auranofin (Fig. 5) and the high dose of GST (Fig. 6). Microsomal AHH activities were unaltered by either auranofin or GST treatment (Table 2).

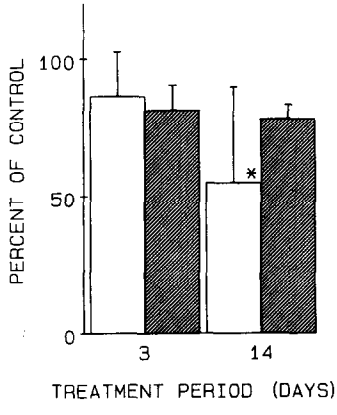

Fig. 4. GST effects on hepatic BPND activity. BPND activity was determined following 3 or 14 days of GST administration at doses of 1.2 (□) and 5.8 (▨) mg/kg. Data are presented as the percent of the corresponding control values (3 days, 0.98 ± 0.24; 14 days, 1.62 ± 0.19 nmoles HOCH/min/mg protein) with each point representing the mean ± S.E.M. (N = 4). Key: (*) significantly different from control at $P < 0.05$.

Three days of treatment with auranofin at all doses studied and the low dose of GST had no effect on ALA synthetase activity (Table 3). However, the high dose of GST produced a significant decrease (50% of control) in ALA synthetase activity (Table 3). Hepatic heme oxygenase activities were increased (14–40%) by auranofin and GST after 3 days of treatment (Table 3).

Renal variables were also examined after 3 days of treatment. Kidney to body weight ratios, renal P-450 content and ECOD activities were unaffected by 3 days of treatment with either auranofin or GST (Table 4). Renal ALA synthetase activity was unchanged by either treatment at 3 days (Table 5). Heme oxygenase activity was increased to 480% of control values by 40 mg/kg of auranofin, but activi-

Table 2. Effects of repeated auranofin and GST treatment on microsomal AHH activity

Treatment*	AHH activity [nmoles 3 + 9-OH B[*a*]P · (mg protein)$^{-1}$ · min^{-1}] 3 days	14 days
Control	1.51 ± 0.31†	3.06 ± 0.30
Auranofin (2 mg/kg/day)	1.99 ± 0.38	1.42 ± 0.24‡
Auranofin (9 mg/kg/day)	1.88 ± 0.09	2.42 ± 0.55
Auranofin (40 mg/kg/day)	1.56 ± 0.25	1.96 ± 1.52§
GST (1.2 mg/kg/day)	2.21 ± 0.13	3.16 ± 0.27
GST (5.8 mg/kg/day)	1.40 ± 0.32	2.93 ± 0.11

* Rats were treated daily for 3 or 14 days and killed 24 hr after the final dose.

† Data are expressed as mean ± S.E. (N = 4).

‡ Significantly different from the matched control at $P < 0.05$.

§ Data are expressed as mean ± S.D. (N = 2).

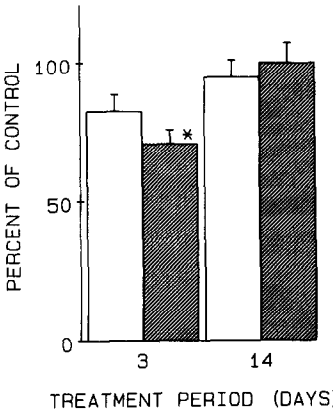

Fig. 6. GST effects on hepatic ECOD activity. ECOD activity was determined following 3 or 14 days of GST administration at doses of 1.2 (□) and 5.8 (▨) mg/kg. Data are presented as the percent of the corresponding control values (3 days, 1.22 ± 0.18; 14 days, 0.91 ± 0.07 nmoles 7-OH coumarin/min/mg protein) with each point representing the mean ± S.E.M. (N = 4). Key: (*) significantly different from control at $P < 0.05$.

ties in the 2 and 9 mg/kg groups and GST groups were unaltered.

Fourteen-day studies. Administration of auranofin for 14 days produced a decrease in the rate of body weight gain (data not shown). No effect was observed with GST. Liver to body weight ratios were markedly decreased in the high-dose auranofin group but were unchanged in the other treatment groups (data not shown). Hepatic P-450 content was not altered significantly after 14 days of treatment with auranofin (Fig. 1) or GST (Fig. 2). Treatment with auranofin at 2, 9, and 40 mg/kg significantly reduced hepatic BPND (35–50% of control) activity (Fig. 3) as did the low dose of GST (55% control) (Fig. 4). ECOD activity was reduced only in the auranofin low-dose group (Fig. 5). Decreases in AHH activity also were limited to the auranofin low-dose group (Table 2). ALA synthetase and heme oxygenase activities were increased, 56 and 200%, respectively, in the auranofin low-dose group but were unaltered by the higher auranofin doses or by either GST dose at 14 days (Table 3).

After 14 days of high-dose auranofin treatment, kidney to body weight ratios were increased (data not shown). Renal P-450 content was reduced markedly (15% of control) in the auranofin high-dose group but was unchanged in the other groups (Table 4). Fourteen days of auranofin or GST treatment did not alter ECOD activity. ALA synthetase activity was increased significantly (289% of control) in the high-dose auranofin group, whereas this activity was unchanged in the other treatment groups (Table 5). Extensive, dose-dependent increases in renal heme oxygenase activities were observed after 14 days of treatment with either auranofin or GST (Table 5).

DISCUSSION

The studies demonstrate that repeated oral administration of auranofin or parenteral administration of myochrysine produced dose- and time-dependent changes in hepatic and renal P-450 content and associated enzyme activities in rats. Both agents reduced hepatic P-450 content after 3 days of treatment, but P-450 content returned to control values with continued treatment for 14 days. Corresponding decreases in ECOD activity, a P-448-associated activity [18], were observed at 3 days. However, another P-448-associated activity, AHH, and a P-450-associated activity, BPND, were unaltered at 3 days. In contrast to the 3-day results, P-450 content had returned to control values after 14 days of treatment, while BPND activity was decreased by both auranofin and GST at this time. Since one P-448-associated isozyme activity (ECOD)

Table 3. Auranofin and GST effects on hepatic ALA synthetase and heme oxygenase

	ALA synthetase [pmoles ALA · hr^{-1} · (mg protein)$^{-1}$]		Heme oxygenase [nmoles bilirubin · hr^{-1} · (mg protein)$^{-1}$]	
Treatment*	3 days	14 days	3 days	14 days
Control	92 ± 13	72 ± 8	1.01 ± 0.10	1.62 ± 0.13
Auranofin (2 mg/kg	80 ± 40	113 ± 13†	1.14 ± 0.13	3.27 ± 0.83
Auranofin (9 mg/kg)	67 ± 10	55 ± 13	1.44 ± 0.12†	2.43 ± 0.77
Auranofin (40 mg/kg)	67 ± 3	45 ± 9‡	1.41 ± 0.17†	2.14 ± 0.57‡
GST (1.2 mg/kg)	67 ± 70	92 ± 3	1.19 ± 0.15	1.81 ± 0.03
GST (5.8 mg/kg)	42 ± 11†	82 ± 14§	1.41 ± 0.01†	1.97 ± 0.01

Data are expressed as mean ± S.E.M. (N = 4).

* Rats were treated daily for 3 or 14 days with auranofin (p.o.) or GST (i.p.) and were killed 24 hr after the final dose.

† Significantly different from corresponding control at $P < 0.05$.

‡ N = 2, mean ± S.D.

§ N = 3.

Table 4. Auranofin and GST effects on renal weight, P-450 content, and ECOD activity

Treatment*	(Kidney wt/Body wt) × 100		Cytochrome P-450 (nmoles/mg protein)		ECOD [nmoles 7-OH coumarin $min^{-1} \cdot (mg\ protein)^{-1}$]	
	3 days	14 days	3 days	14 days	3 days	14 days
Control	0.86 ± 0.02	0.75 ± 0.03	0.046 ± 0.019	0.076 ± 0.017	9.76 ± 2.51	18.73 ± 3.23
Auranofin (2 mg/kg)	0.85 ± 0.02	0.84 ± 0.02	0.063 ± 0.024	0.073 ± 0.015	10.86 ± 3.72	28.96 ± 6.24
Auranofin (9 mg/kg)	0.87 ± 0.03	0.85 ± 0.04	0.036 ± 0.007	0.067 ± 0.006	11.08 ± 1.33	14.16 ± 1.04
Auranofin (40 mg/kg)	0.88 ± 0.04	0.93 ± 0.01†,‡	0.053 ± 0.016	0.011 ± 0.014†,‡	14.93 ± 2.18	10.63 ± 0.53
GST (1.2 mg/kg)	0.87 ± 0.03	0.76 ± 0.05	0.065 ± 0.013	0.067 ± 0.007	15.80 ± 0.80	19.17 ± 0.83
GST (5.8 mg/kg)	0.81 ± 0.01	0.92 ± 0.02	0.060 ± 0.010	0.055 ± 0.011	13.63 ± 1.26	14. 64 ± 0.25

Data are expressed as mean ± S.E.M. (N = 4).
* Rats were administered auranofin (p.o.) or GST (i.p.) daily for 3 or 14 days and killed 24 hr after the final dose.
† Significantly different from corresponding control at $P < 0.05$.
‡ N = 2, mean ± S.D.

rapidly decreased and returned to control values by 14 days while another P-448 (AHH) and a P-450 (BPND) isozyme-associated activity were not affected until later (14 days), it may be speculated that selected isozymes are lost more rapidly and others return at a slower rate (isozyme specific turnover rates). Unlike the reported [7] effects of single high doses of GST on hepatic P-450 content and associated enzyme activities, high single oral doses of auranofin did not alter hepatic P-450.

The observed increases in heme oxygenase may be associated with the decreases observed in P-450 content. Heme oxygenase activity was elevated after 3 days of auranofin and GST administration, whereas ALA synthetase activity was generally unaltered. This translates to a potential increase in heme catabolism in the absence of a compensatory increase in heme anabolism. This would be expected to produce a net loss in hepatic heme content. These observations and this interpretation are consistent with results from studies [7] using single high doses of GST and in general agreement with previously reported effects of heavy metals on hepatic hemoproteins [8–12]. The reversal of the effects on P-450 at 14 days also is consistent with reports of tolerance observed after repeated dosing with gold [28], and the lack of effects on P-450 following 20 days of GST administration [7]. One possible mechanism of tolerance is the induction of the metal binding protein metallothionein (MT) [19]. It has been demonstrated that gold treatment can induce MT [30–32] which would increase hepatocyte capacity to sequester gold and reduce the cytosolic free gold concentration. Thus, it can be speculated that the resulting decrease in gold concentration would reduce the gold stimulation of heme oxygenase activity, leading to a restoration of balanced heme anabolism and catabolism.

The renal effects observed were similar to the

Table 5. Auranofin and GST effects on renal ALA synthetase and heme oxygenase

Treatment*	ALA synthetase [pmoles ALA · hr^{-1} · (mg protein)$^{-1}$]		Heme oxygenase [nmoles bilirubin · hr^{-1} · (mg protein)$^{-1}$]	
	3 days	14 days	3 days	14 days
Control	74 ± 14	132 ± 47	0.46 ± 0.18	0.46 ± 0.18
Auranofin (2 mg/kg)	80 ± 43	106 ± 48	0.69 ± 0.25	3.55 ± 1.01†
Auranofin (9 mg/kg)	27 ± 10	212 ± 21	1.23 ± 0.80	4.44 ± 1.04†
Auranofin (40 mg/kg)	60 ± 34	382 ± 61†,‡	2.24 ± 0.66†	12.28 ± 4.70†,‡
GST (1.2 mg/kg)	92 ± 28	204 ± 29	0.50 ± 0.37	3.03 ± 0.78†
GST (5.8 mg/kg)	100 ± 19	242 ± 45	0.39 ± 0.24	6.28 ± 1.61†

Data are expressed as mean ± S.E.M. (N = 4).
* Rats were treated daily for 3 or 14 days with auranofin (p.o.) or GST (i.p.) and were killed 24 hr after the final dose.
† Significantly different from corresponding control at $P < 0.05$.
‡ N = 2, mean ± S.D.

hepatic effects in that reductions in P-450 content were observed. However, unlike the liver, renal heme oxygenase activity increases were apparent prior to alterations in P-450 content, and changes in P-450 content were not noted until 14 days of treatment. Furthermore, these effects were only present in the animals treated with the high doses of auranofin or GST. Although reversal of the high-dose auranofin effects on P-450 were not observed in the kidney, the increases in ALA synthetase activity observed at 14 days suggest that P-450 content might return to control values with continued dosing. As with liver, the kidney results are consistent with previous literature reports with respect to GST effects [7] and to heavy metal induced alterations in heme metabolism [9–12]. The lag time prior to the onset of effect in the kidney, relative to the liver, may be the result of differences in renal gold accumulation kinetics [33] and basal MT content between kidney and liver [28]. Higher renal MT concentrations (five to ten times greater than liver) would increase the kidney gold binding capacity and the time required for intracellular free gold concentrations to attain levels necessary to affect heme oxygenase activity. Similar to the tolerance mechanism proposed for the liver, elevated renal gold concentrations would stimulate MT induction [29–31] leading to reduced free values and decreased heme oxygenase effects.

In conclusion, these studies demonstrate that administration of auranofin or GST to rats produced transient and reversible decreases in hepatic and renal P-450 content and associated enzyme activities. These changes were concurrent with increases in heme oxygenase activity, indicating that gold-induced alterations in heme metabolism could account for the observed effects. Finally, these results demonstrate that administration of auranofin or GST to rats at twenty times the human dose produced classical heavy metal effects on heme metabolism and cytochrome P-450 content which are reversible.

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