POTENT HEME-DEGRADING ACTION OF ANTIMONY AND ANTIMONY-CONTAINING PARASITICIDAL AGENTS*

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Antimony compounds have been employed medicinally for over 4,000 years. In Roman times antimony-rich vessels were used to store wine which was then drunk to induce vomiting; while in the middle ages antimony was employed as a panacea against the plague. In recent times antimony compounds have been employed therapeutically against a number of parasitic diseases including trypanosomiasis, leishmaniasis, bilharziasis, and schistosomiasis (1).

Both tri- and pentavalent antimony compounds have been administered for parasiticidal effects; however the former have proved more effective, probably because they are freely transferable through the erythrocyte membrane whereas the pentavalent forms are not (2). In circulating blood antimony is removed extremely rapidly, partly because of its rapid excretion in the urine (especially the pentavalent form) and partly because of its uptake by different body tissues (2). This has resulted in the administration of antimony over extended time periods, lasting many weeks, in the treatment of parasitic diseases (3). Liver injury, manifested by fatty degeneration (4) and jaundice (5), has been associated with such prolonged antimony treatment.

Recent studies from this laboratory have demonstrated that a number of transition elements and heavy metals (6–8) are capable of increasing the *de novo* synthesis of heme oxygenase, the rate-limiting enzyme in the oxidative metabolism of heme to bile pigment (9, 10). Included among the metals that are potent inducers of heme oxygenase are environmental pollutants such as tin (11), cobalt (8), cadmium (8), and platinum (12) and metals such as gold (13) and iron (8), which are used therapeutically in man. Because of the extensive use of antimony as a parasiticidal, agent, it seemed of interest to assess the ability of this metal and also of antimony-containing drugs to induce heme oxygenase. The results of this study show that trivalent antimony is an extremely potent inducer of heme oxygenase in liver and kidney, whereas pentavalent antimony is a poor inducer of this enzyme. Antimony-containing parasiticidal agents also greatly enhance the rates of heme degradation in these tissues.

Materials and Methods

Materials. Male Sprague-Dawley rats, 175–225 g, purchased from Holtzman Co., Madison, Wis. were used throughout this study. All antimony salts were purchased from Fisher Scientific Co., Pittsburgh, Pa., except antimony sodium dimercaptosuccinate, which was a gift of Hoffmann-La Roche, Inc., Nutley, N. J. Antimony sodium dimercaptosuccinate (Astiban), antimony potassium tartrate (tartar emetic) and antimony sodium gluconate (Pentostam) were the parasiticidal agents examined in this study. All other chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.

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Treatment of Animals. Rats were injected at subcutaneous sites with either zinc or cobalt chloride and a variety of antimony salts at doses up to 250 μmol/kg. Control rats received an equivalent volume of normal saline. Desferoxamine mesylate (Desferal mesylate; Ciba-Geigy Corp., Summit, N. J.) which is used therapeutically to chelate iron was injected intraperitoneally at a dose of 1 gm/kg. The rats were permitted water but were starved for 16 h after metal administration before being killed by decapitation. Livers were perfused *in situ* with ice-cold saline and were then removed and homogenized, as were other tissues, in 3 vol 0.1 M potassium phosphate, pH 7.4, containing 0.25 M sucrose. The homogenate was centrifuged at 9,000 *g* for 20 min. The precipitate was washed twice with 0.1 M potassium phosphate, pH 7.4, before assaying for δ-aminolevulinate synthase (ALAS)[1] activity. The 9,000-*g* supernate was centrifuged at 100,000 *g* for 60 min; the cytosol was employed as a source of biliverdin reductase; and the microsomal pellet was washed once with and resuspended in 0.1 M potassium phosphate buffer, pH 7.4.

Assays

ENZYME ACTIVITIES. The activities of ALAS (14), aniline hydroxylase (15), ethylmorphine demethylase (7), and heme oxygenase (16) were assayed as previously described. The formaldehyde produced in the ethylmorphine demethylase assay was measured by the method of Nash (17). Bilirubin produced in the heme oxygenase assay was calculated using an extinction coefficient of 40 mM^{-1} cm^{-1} between 464 and 530 nm.

PROTEIN ASSAY. Protein concentration was determined by the method of Lowry (18) using crystalline bovine serum albumin as standard.

SPECTRAL STUDIES. Cytochrome P-450 was measured using sodium dithionite as a reducing agent and using an extinction coefficient of 91 mM^{-1} cm^{-1} between 450 and 490 nm (19). Microsomal heme concentration was determined by the pyridine hemochromogen method using an extinction coefficient of 20.7 mM^{-1} cm^{-1} between 557 and 540 nm (20). An Aminco-Chance DW2A spectrophotometer (American Instrument Co., Silver Spring, Md.) in the split beam mode was employed for the spectral studies.

Statistical Analysis. The data were analyzed by the standard *t* test and the value of $P < 0.05$ was regarded as denoting significance.

Results

Dose-Response Curves of Antimony Induction of Heme Oxygenase in Liver and Kidney. Trivalent antimony was found to be an extremely potent inducer of heme oxygenase in liver (Fig. 1). At an antimony dose of 25 μmol/kg (3.0 mg/kg) heme oxygenase activity was increased fivefold when measured at 16 h; by comparison a comparable induction of the enzyme requires severalfold larger amounts of other inducing metals (8, 21). A maximum induction effect, (10-fold greater than control levels), was achieved with an antimony dose of 125 μmol/kg (15.0 mg/kg). Administration of pentavalent antimony also increased hepatic heme oxygenase activity, but the maximum levels obtained were threefold higher than control levels and were reached only after administration of a substantially higher (250 μmol/kg) dose of the metal. The dose-response of heme oxygenase induction in kidney (Fig. 1) to trivalent antimony administration was strikingly similar to that in liver in that enzyme levels were markedly increased at the lowest levels of antimony administered and reached a maximum of ~11-fold above control levels at a dose of 125 μmol/kg. Pentavalent antimony was not a potent inducer of heme oxygenase in kidney, a maximum induction of 1.5-fold being obtained at a dose of 125 μmol/kg (Fig. 1).

Time-Course of Trivalent Antimony Induction of Heme Oxygenase and Concomitant Perturbations of Heme Metabolism in Liver and Kidney. The levels of heme oxygenase in liver did not

[1] *Abbreviation used in this paper:* ALAS, δ-aminolevulinate synthase.

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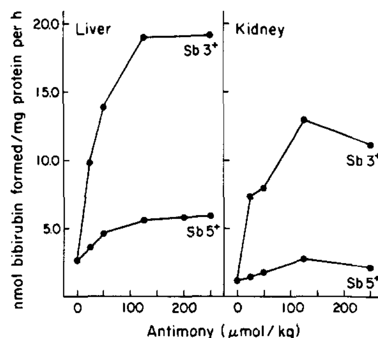

FIG. 1. The dose-response curve of tri- and pentavalent antimony administration on heme oxygenase induction in liver and kidney. The animals (four to six per point) were treated with the metal chlorides as described in Materials and Methods.

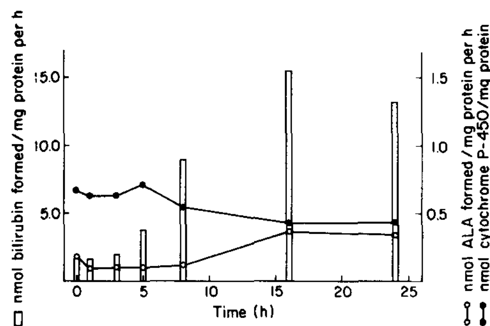

FIG. 2. The time-course of response in liver to trivalent antimony chloride administered at a dose of 250 μmol/kg. Assays were carried out as described in Materials and Methods. Three animals were used at each time point.

increase significantly during the first 3 h after antimony administration, but by 5 h there was a rise of more than twofold (Fig. 2). The enzyme activity increased dramatically thereafter, reaching a maximum at 16 h ~10-fold higher than the initial level and remaining highly elevated at 24 h. ALAS activity decreased by 30% 1 h after antimony administration. This enzyme activity remained depressed up to 8 h before exhibiting a rebound increase in activity at 16 h similar to that previously described with cobalt administration (7). This elevated level of ALAS activity was then maintained through the 24-h time period. The level of cytochrome P-450 did not change significantly until 8 h after antimony administration when a decrease of 18% was evident; by 16 h the cytochrome P-450 level had decreased by almost 40%. Microsomal heme levels exhibited similar changes (data not shown). The level of cytochrome P-450 remained low at 24 h despite the increased level of ALAS; presumably this was because heme oxygenase activity was still some 10-fold above normal at this time. Concomitant with the low cytochrome P-450 level, aniline hydroxylase activity was decreased by almost 40% at 16 h; cytochrome b_5 content remained unchanged (results not shown) probably because of its long half-life in contrast to that of cytochrome P-450. In kidney, heme oxygenase activity did not change significantly during the initial 3 h after antimony administration (Fig. 3); however, the enzyme level had doubled by 5 h and continued to rise rapidly reaching

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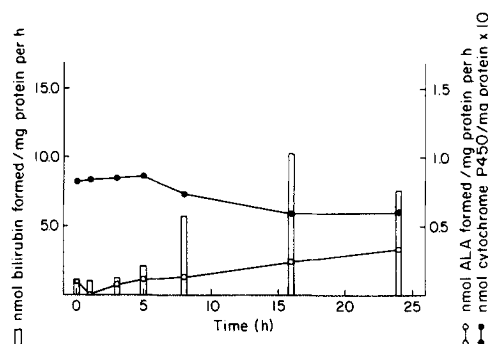

FIG. 3. The time-course of response to trivalent antimony chloride in kidney. The conditions were identical to those described in Fig. 2.

TABLE I

Effect of Trivalent Antimony on Heme Oxygenase and ALAS Activity In Vitro

Antimony	Heme oxygenase activity (nmol bilirubin formed/ mg protein per h)		ALAS activity (nmol ALA formed/mg protein per h)	
	Liver	Kidney	Liver	Kidney
mM				
0	2.67	1.04	0.110	0.086
0.10	2.89	1.70	0.048	0.084
0.25	2.89	1.51	0.038	0.066
0.50	3.02	1.47	0.037	0.063
1.00	2.95	1.17	0.029	0.042

Heme oxygenase and ALAS were prepared and assayed as described in Materials and Methods. The assays were done in duplicate and are the average of three experiments.

a maximum 10-fold higher than the initial level by 16 h. High levels of renal heme oxygenase were maintained for at least 24 h after antimony administration. ALAS activity in kidney dropped sharply after antimony administration and did not return to the control level until 8 h, after which the enzyme activity reached a level twofold higher than control at 16 h and almost threefold higher at 24 h. Cytochrome P-450 content remained constant for the initial 5 h before decreasing by 30% at 16 h.

Effect of Trivalent Antimony In Vitro on Heme Oxygenase and ALAS. A series of concentrations (up to 1 mM) of trivalent antimony were tested in vitro for their effect on heme oxygenase and ALAS activities isolated from liver and kidney (Table I). Antimony $\leq$1 mM had no effect on hepatic heme oxygenase activity in vitro, but 0.1 mM antimony increased renal heme oxygenase activity by 60%; this increase in activity declined with increasing antimony concentration, although at 1 mM antimony renal heme oxygenase activity was still greater than the control level. In vitro antimony, even at a concentration of 0.1 mM, was a potent inhibitor (~60%) of ALAS activity isolated from liver; inhibition of ALAS activity was significantly greater with the hepatic enzyme than with the renal enzyme.

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TABLE II

Effect of Various Antimony Salts on Heme Oxygenase Induction and the Concomitant Perturbations in Heme Metabolism in Liver

	Heme oxygenase (nmol bilirubin formed/mg protein per h)	ALAS (nmol ALA/mg protein per h)	Cytochrome P-450	Microsomal heme	Ethylmorphine demethylase (μmol/mg protein per h)	Aniline hydroxylase (nmol *p*-aminophenol/mg protein per h)
			nmol/mg	*nmol/mg*		
Saline	2.52 ± 0.14	0.170 ± 0.01	0.73 ± 0.03	1.64 ± 0.06	0.408 ± 0.06	98.28 ± 7.22
Antimony* trichloride	15.35 ± 0.92‡	0.295 ± 0.02‡	0.54 ± 0.01‡	1.39 ± 0.04‡	0.253 ± 0.04‡	75.26 ± 5.01
Antimony* potassium tartarate (tartar emetic)	10.57 ± 1.95‡	0.478 ± 0.05‡	0.47 ± 0.03‡	1.24 ± 0.07‡	0.205 ± 0.04‡	48.70 ± 4.13‡
Antimony* sodium dimercaptosuccinate (Astiban)	9.53 ± 0.20‡	0.378 ± 0.05‡	0.66 ± 0.01	1.54 ± 0.01	0.419 ± 0.05	91.20 ± 8.01
Antimony pentachloride	4.67 ± 0.36‡	0.178 ± 0.08	0.70 ± 0.04	1.48 ± 0.04	0.412 ± 0.05	98.28 ± 1.11
Sodium stibogluconate (Pentostam)	2.58 ± 0.44	0.155 ± 0.02	0.73 ± 0.01	1.58 ± 0.03	0.392 ± 0.06	97.39 ± 8.14

The concentration of antimony used was 82 μmol/kg (10 mg/kg). The animals (four to six per compound) were treated as described in Materials and Methods.

* Trivalent antimony.

‡ $P < 0.05$, significantly different from the control value.

TABLE III

Effect of Blocking Agents on Antimony and Cobalt Induction of Heme Oxygenase and Associated Perturbations in ALAS Activity and Cytochrome P-450 Content

Treatment	Heme oxygenase (nmol bilurubin/mg protein per h)	ALAS (nmol ALA/mg protein per h)	Cytochrome P-450
			nmol/mg
Saline	2.66 ± 0.13	0.154 ± 0.03	0.69 ± 0.05
Sb^{3+}	17.23 ± 1.92	0.297 ± 0.03	0.39 ± 0.03
$Sb^{3+} + Zn^{2+}$	14.33 ± 1.12	0.311 ± 0.04	0.43 ± 0.05
Sb^{3+}-cysteine complex	17.97 ± 1.28	0.214 ± 0.01	0.33 ± 0.03
Cysteine (oral) before Sb^{3+}	26.22 ± 0.86	0.252 ± 0.02	0.34 ± 0.02
Co^{2+}	13.87 ± 0.66	0.593 ± 0.05	0.48 ± 0.04
$Co^{2+} + Zn^{2+}$	7.68 ± 0.88*	0.365 ± 0.03*	0.53 ± 0.06
Co^{2+}-cysteine complex	2.63 ± 0.33*	0.293 ± 0.02*	0.68 ± 0.02*
Cysteine (oral) before Co^{2+}	3.18 ± 0.25*	0.185 ± 0.05*	0.72 ± 0.02*

The concentration of antimony, cobalt, and zinc chlorides used was 250 μmol/kg. Cysteine (1–2 ml of a 1 M solution) was administered by gavage 15 min before the inducing metal. The metals were complexed with cysteine at a molar ratio of 1:4 for subcutaneous administration as a complex.

* $P < 0.05$, significantly different from the control value.

Effects of Various Tri- and Pentavalent Salts of Antimony on Heme Oxygenase Induction and Associated Parameters of Heme Metabolism in Liver. The antimony salts, including parasiticidal agents, examined were administered to rats at a dose of 10 mg antimony/kg which is in the dose range generally employed therapeutically in humans. It is evident from Table II that trivalent antimony salts, including two of the parasiticidal drugs tested, are potent inducers of heme oxygenase in liver, whereas pentavalent antimony salts are not. This effect appears independent of the salt employed.

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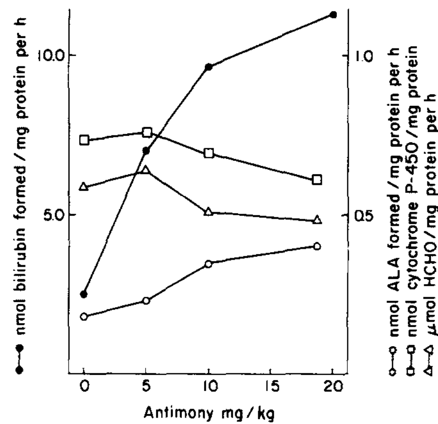

Fig. 4. The dose-response curve in liver of various parameters of heme metabolism to antimony sodium dimercaptosuccinate. Four to six animals were used for each point.

Table IV

Effect of Desferoxamine on Perturbations in Heme Metabolism Produced by Antimony and Cobalt

Treatment	Heme oxygenase (nmol bilirubin/mg protein per h)	ALAS (nmol ALA/mg protein per h)	Cytochrome P-450	Microsomal heme
			nmol/mg	*nmol/mg*
Saline	2.42 ± 0.20	0.163 ± 0.01	0.89 ± 0.03	1.92 ± 0.04
Desferoxamine	3.89 ± 0.31	0.186 ± 0.03	0.76 ± 0.04	1.66 ± 0.05
Sb^{3+}	9.37 ± 0.32	0.458 ± 0.03	0.75 ± 0.04	1.71 ± 0.06
Sb^{3+} + desferoxamine	7.00 ± 0.28	0.188 ± 0.02*	0.64 ± 0.04	1.53 ± 0.06
Co^{2+}	16.05 ± 0.80	0.425 ± 0.02	0.68 ± 0.03	1.53 ± 0.04
Co^{2+} + desferoxamine	15.11 ± 1.07	0.200 ± 0.02*	0.47 ± 0.04*	1.12 ± 0.05*

Antimony was administered subcutaneously as sodium dimercaptosuccinate at a concentration of 82 µmol/kg (10 mg/kg). Cobalt was administered as $CoCl_2 \cdot 6H_2O$ subcutaneously at a concentration of 250 µmol/kg. Desferoxamine was administered intraperitoneally at a dose of 1 gm/kg.

* $P < 0.05$, significantly different from the control value.

Associated with the induction of heme oxygenase was a significant increase in ALAS activity as measured at 16 h (see Fig. 2). This represents the rebound increase in ALAS shown in Fig. 2 and previously reported as a late enzyme response to other inorganic metals (7, 12). The levels of cytochrome P-450 and microsomal heme decreased concomitantly with the increased levels of heme oxygenase, although these changes were more pronounced with the chloride and the potassium tartarate salts of trivalent antimony than with the sodium dimercaptosuccinate salt. Similarly, the decreases in cytochrome P-450-dependent ethylmorphine demethylase and aniline hydroxylase activities were most evident after administration of antimony trichloride and antimony potassium tartarate.

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In kidney, trivalent salts including the trivalent antimony parasiticidal agents were also more potent inducers of heme oxygenase than the pentavalent salts (Fig. 1). This effect was independent of the salt used. The other perturbations in heme metabolism were similar to those described for liver.

Effect of Cysteine and Zinc on Heme Oxygenase Induction by Antimony and Cobalt. Both trivalent antimony and divalent cobalt are potent inducers of heme oxygenase in liver (Figs. 1 and 2 and Table III). Administration of cysteine by gavage or as cysteine complexed with antimony did not prevent heme oxygenase induction or the concomitant decrease in cytochrome P-450 levels and the changes in ALAS activity. The changes in heme metabolism associated with cobalt in vivo were prevented, as previously described (8), by either pretreatment with cysteine or administration of a cysteine cobalt complex under identical conditions to those used for antimony. Similarly, zinc has been shown to substantially block the induction of heme oxygenase in liver by cobalt (22). However zinc was unable to block the antimony-mediated induction of heme oxygenase in liver and the concomitant perturbations in heme metabolism (Table III).

Dose-Response of Various Parameters of Heme Metabolism to Antimony Sodium Dimercaptosuccinate (Astiban) in Liver. Heme oxygenase activity increased with increasing concentration of this antimony derivative when measured 16 h after metal administration (Fig. 4). A dose of 5 mg/kg antimony resulted in an almost twofold increase in enzyme activity, whereas a dose of 20 mg/kg caused an ~fourfold increase. ALAS activity exhibited a similar dose-response reflected in increased ALAS activity at 16 h associated with increasing dose of metal administered. Levels of cytochrome P-450 declined with increasing amounts of antimony, as did ethylmorphine demethylase activity. A similar series of changes was observed in kidney (data not shown).

Effect of Desferoxamine (Desferal Mesylate) on Various Parameters of Heme Metabolism Mediated by Antimony and Cobalt. Trivalent antimony (10 mg/kg) induced hepatic heme oxygenase almost fourfold above control level (Table IV). Concomitant with this increase in heme oxygenase was a substantial rebound increase in ALAS at 16 h (Table IV). Simultaneous administration of desferoxamine did not markedly alter the extent of heme oxygenase induction by antimony, but completely blocked the rebound increase in ALAS. The decreases in levels of cytochrome P-450 and microsomal heme on antimony administration were more pronounced on simultaneous desferoxamine treatment. Identical results were observed with cobalt in that desferoxamine did not block heme oxygenase induction, but prevented the rebound increase of ALAS activity and, in addition, further lowered the contents of cytochrome P-450 and microsomal heme.

Discussion

This study demonstrates that antimony and antimony-containing parasiticidal agents have a potent ability to induce the microsomal enzyme heme oxygenase in both liver and kidney (Fig. 1 and Table II) and, consequently, to enhance the rates of heme degradation in these tissues. The potent ability of trivalent antimony to alter these aspects of heme metabolism is independent of the salt used (Table II) and does not appear from in vitro studies to be a direct activation of the enzyme. The extent of enzyme induction in liver is comparable to that produced by cobalt, but is obtained

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with a dose one-half that reported for the latter element (6). Pentavalent antimony is a weak inducer of heme oxygenase in both organs studied (Fig. 1).

Associated with the increase in heme oxygenase activity produced by trivalent antimony is a substantial decrease in cytochrome P-450 (Figs. 2 and 3 and Table II) and heme contents (Table II) and a marked decrease in the activities of the cytochrome P-450-dependent drug metabolizing enzymes ethylmorphine demethylase and aniline hydroxylase (Table II). The sequential changes—an initial inhibition and a rebound increase in ALAS activity—that usually occur with cobalt administration (7) also occur after antimony administration (Figs. 2 and 3). Thus, trivalent antimony through its potent heme oxygenase-inducing action leads to deleterious perturbations in heme metabolism reflected in significant impairment of the cytochrome P-450-dependent mixed function oxidase system.

The potent ability of trivalent as compared with pentavalent antimony to induce heme oxygenase is of interest because trivalent antimony compounds have proved more effective than pentavalent antimony compounds as parasiticidal agents (23). The reason for this is not clear but trivalent compounds tend to concentrate in erythrocytes, whereas pentavalent compounds do not; high levels of both forms are present in the plasma and are thought to be bound to plasma proteins (2). Trivalent antimony passes freely through the erythrocyte membrane (2) and as such may be the more available form of antimony.

The normal therapeutic use of antimony compounds involves a series of injections generally lasting several weeks in duration (3). In this study, a single dose, in animals, of the usual single therapeutic dose (10 mg/kg) of trivalent antimony in man was shown to produce a four- to sixfold increase in hepatic heme oxygenase activity (Table II). Repeated administration of antimony would thus be expected to lead to sustained high levels of heme oxygenase activity (7) and prolonged decreases in cellular contents of cytochrome P-450 and other hemoproteins (7, 11). Thus the intensive antimony therapeutic regimens employed in man could be seriously detrimental to certain heme-dependent cellular functions in the absence of adaptive or compensatory mechanisms which would minimize the toxicity of the metal.

There exist several possible defense mechanisms against the toxic effects of metals on heme metabolism. We have shown that complexing metals with glutathione or cysteine in vitro or administration of cysteine by gavage protect against the toxic effects of cobalt, nickel, and platinum on heme metabolism (8, 24). Manganese in kidney and zinc in liver and kidney when administered either simultaneously or before a heme-oxygenase-inducing metal have been shown to reduce or abolish the metal-mediated induction of the enzyme and the associated decreases of cellular heme and hemoprotein contents (22). However, antimony-mediated induction of heme oxygenase as shown in this study was not prevented either by the simultaneous administration of zinc or by the use of cysteine in vitro or in vivo (Table III). Cobalt induction of heme oxygenase by comparison was largely blocked by zinc treatment and entirely blocked by cysteine treatment (Table III). The explanation for the inability of either zinc or cysteine to block antimony induction of heme oxygenase is not evident, but the finding, with cysteine, is of particular interest because dimethylcysteine is known to reduce the toxic, but not the therapeutic, effects of drugs that contain arsenic and antimony (25). Arsenic, like antimony, induces heme oxygenase in both liver and kidney (data not shown).

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It was of interest that both in the case of antimony and cobalt, desferoxamine, a chelator of trivalent metals, especially iron, was unable to significantly diminish the induction of heme oxygenase but almost entirely prevented the late rebound increase in ALAS activity (Table IV) caused by both elements. These findings raise the possibility that iron, released during the metal-induced heme degradation process may, as has been shown in vivo (26), participate in a synergistic induction process for ALAS in some manner, thus accounting, in part, for the rebound increase of this enzyme. This rebound takes place when the original inducer metal has presumably been excreted from the liver or kidney and thus cannot continue to repress the synthesis of ALAS (21). However, it should be noted that iron itself not only induces heme oxygenase (8, 27), but also produces the early transient decline in ALAS activity associated with the induction of heme oxygenase by many other metals such as cobalt or antimony.

Parasiticidal therapy with antimony compounds is generally prolonged (3) and jaundice frequently occurs (5); the duration of treatment can in some instances extend for >25 wk (28) at high doses of the metal-containing drugs. It is possible therefore that the jaundice described during such extensive treatment periods results not only from direct hepatocellular toxicity but also, in part, from the long sustained and marked enhancement of heme degradation mediated by the antimony in the parasiticidal agent. The cellular depletions of heme and hemoproteins, which could occur during such prolonged therapeutic regimens, would be expected to impair significantly those cellular functions dependent on respiratory cytochromes and the chemical detoxification processes known to be dependent on an intact cytochrome P-450 system. These effects of antimony resulting from prolonged induction of heme oxygenase would have such deleterious effects if the actions of the metal on the enzyme could not be blocked or if the perturbation of heme metabolism could not be compensated by some mechanism, perhaps similar to that we have described in chronic, genetically dependent hemolysis in mice homozygous for the nb (normoblastosis) gene, i.e., a marked and sustained increase in heme synthetic activity (14).

The mechanism of the parasiticidal action of antimony has not been entirely clarified, but is thought to be a result of a preferential uptake of the metal by the parasite resulting in an inhibition of anaerobic glycolysis—specifically at the site of phosphofructokinase activity (29). The studies described here indicate that antimony is also a potent inducer of the enzyme heme oxygenase, and as a consequence of this action, can substantially increase the rate of heme degradation. The relation between these distinct biochemical actions of antimony is not known.

Heme has been shown to be essential for the growth of certain trypanosomatidae (30). Cytochromes a_3 and *o* have been reported in cultured forms of *Trypanosoma mega* (31), cytochromes aa_3, *b*, and *c* in *Trypanosoma brucei* (32), and an inducible cytochrome, P-450, has been described in *Trypanosoma cruzi* (33). In *Crithidia lucilia*, catalase has been proposed to act as a reservoir for heme (34). If the heme-degradative actions of drugs containing antimony and arsenic have some relation to their parasiticidal actions, then it might be useful to examine other metals or metalloporphyrins which can markedly degrade heme through prolonged induction of heme oxygenase (35, 36) for possible parasiticidal effects.

Summary

The ability of antimony and antimony-containing parasiticidal agents to enhance the rate of heme degradation in liver and kidney was investigated. Trivalent antimony

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was shown to be an extremely potent inducer of heme oxygenase, the initial and rate-limiting enzyme in heme degradation, in both organs, whereas the pentavalent form was a weak inducer of this enzyme. The ability of antimony to induce heme oxygenase was dose-dependent, independent of the salt used, and not a result of a direct activation of the enzyme in vitro. Concomitant with heme oxygenase induction by antimony, microsomal heme and cytochrome P-450 contents decreased, the cytochrome P-450-dependent mixed function oxidase system was impaired, and δ-aminolevulinate synthase (ALAS), the rate-limiting enzyme of heme synthesis, underwent the sequential changes—initial inhibition followed by rebound induction—usually associated with the administration of transition elements such as cobalt.

Antimony induction of heme oxygenase however, unlike the enzyme induction elicited by cobalt, was not prevented either by cysteine administered orally or as a cysteine metal complex, or by simultaneous zinc administration. Desferoxamine also did not block heme oxygenase induction by antimony, but this chelator did prevent the rebound increase in ALAS activity associated with antimony or cobalt treatment.

Antimony-containing parasiticidal drugs were also potent inducers of heme oxygenase in liver and kidney. The heme degradative action of these drugs may be related in part to the jaundice commonly associated with the prolonged therapeutic use of these agents.

The heme-oxygenase-inducing action of antimony-containing parasiticidal drugs is a newly defined biological property of these compounds. The relation between the parasiticidal and the heme-oxygenase-inducing actions of such drugs is unknown. However, certain parasites contain hemoproteins or require heme compounds during their life cycle. It may therefore be useful to explore the possibility that the heme-degrading and the parasiticidal actions of certain metals or metal-containing therapeutic agents are in some way related.

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