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GLUTATHIONE REDOX STATE, LIPID PEROXIDE LEVELS, AND ACTIVITIES OF GLUTATHIONE ENZYMES IN OLTIPRAZ-TREATED ADULT *SCHISTOSOMA MANSONI*

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Abstract—A decrease in reduced glutathione (GSH) levels in adult *Schistosoma mansoni* exposed *in vitro* to the antischistosomal drug oltipraz (OPZ) (20–60 nM) was accompanied by a significant increase in oxidized glutathione (GSSG) levels. The total glutathione (GSH + GSSG) levels also diminished in drug-treated parasites. The activities of the parasite glutathione peroxidase (GPO), utilizing cumene hydroperoxide as a substrate, and glutathione *S*-transferase (GST), measured 18 hr after *in vitro* incubation with the drug, were elevated significantly, but there were no significant alterations in the activities of the GPO, utilizing H_2O_2, or glutathione reductase (GR). Drug-treated worms showed increased lipid peroxidation. *In vivo*, the proportion of the worms recovered from infected mice given OPZ (100 mg/kg body wt) gradually declined with time, to about 30% of that recovered from infected untreated control mice by day 14 after drug administration, and consisted predominantly of male worms. Accompanying this significant decline in the proportion of worms recovered were significant decreases in the activities of the enzymes GR and GST in drug-exposed worms. On the other hand, a slight initial increase in the GPO activity with cumene hydroperoxide was followed by a return to control values, and the GPO activity with H_2O_2 was decreased only slightly with time. Interestingly, the 4-hydroxyalk-2-enal aldehydes, known products of lipid peroxidation, inhibited the GST reaction with 1-chloro-2,4-dinitrobenzene (CDNB). The OPZ-induced changes in *S. mansoni* could increase parasite susceptibility to oxidative attack by host phagocytes, and are probably linked with the antischistosomal action of the drug *in vivo*.

Oltipraz [4-methyl-5(2-pyrazinyl)-3*H*-1,2-dithiole-3-thione] (OPZ†) shows significant antischistosomal activity in experimental animals [1, 2] and in humans [3–5]. Its mode of action, however, is not well understood. It has been reported to decrease reduced glutathione (GSH) levels of adult *Schistosoma mansoni* exposed to the drug under *in vitro* conditions [6], or *in vivo*, in mice [2, 7]. The drug is also known to inhibit cysteine uptake by the parasites [6, 7], but it does not appear to inhibit parasite glutathione biosynthesis [7]. It is therefore not clear how it acts to reduce schistosome GSH levels. Although the drug has now been withdrawn from further clinical trials because of adverse side effects [8], understanding how it exerts its antischistosomal effects, and particularly how it alters parasite glutathione metabolism, may elucidate unique aspects of schistosome glutathione metabolism and lead to the discovery of new approaches for schistosomiasis chemotherapy and/or immunotherapy.

Glutathione, a tripeptide synthesized intracellularly and present in most living organisms at relatively high concentrations, performs many metabolic functions including protection of cells against oxidant mediated damage, and xenobiotic metabolism and detoxification [9, 10]. The tripeptide exists in the more abundant reduced form (GSH) and the dimeric oxidized form (GSSG), and its metabolic functions are catalyzed by several enzymes including glutathione peroxidase (GPO), glutathione reductase (GR) and glutathione *S*-transferase (GST) [9]. GPO catalyzes the reduction of hydrogen peroxide (H_2O_2) and organic hydroperoxides by GSH, a process leading to GSSG formation; the GSSG is converted to GSH in order to maintain the glutathione redox state by GR, in an NADPH-dependent reaction [9]. GST catalyzes GSH-conjugation with xenobiotics (e.g. drugs) and substances produced endogenously such as the aldehydic products formed during lipid peroxidation [9, 11–13]. GST may also exhibit GPO activity [9, 13].

A number of studies have indicated that chemical-induced GSH depletion in mammalian hepatocytes may be associated with various cellular changes including increased GSSG formation [14–16], increased lipid peroxidation [17, 18], or alterations in the activities of glutathione enzymes [18]. Such events have been linked with the metabolism and/or the toxicity of some of the compounds involved.

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† Abbreviations: OPZ, oltipraz; GSH, reduced glutathione; GSSG, oxidized glutathione; GPO, glutathione peroxidase; H_2O_2, hydrogen peroxide; GR, glutathione reductase; GST, glutathione *S*-transferase; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; DTNB, 5,5′-dithiobis-(2-nitrobenzoic acid); cumene-OOH, cumene hydroperoxide; CDNB, 1-chloro-2,4-dinitrobenzene; DMF, *N*,*N*-dimethylformamide; TBA, thiobarbituric acid; TCA, trichloroacetic acid; BSA, bovine serum albumin; 2-VP, 2-vinylpyridine; HBSS, Hanks' balanced salt solution; HHE, 4-hydroxyhexenal; HNE, 4-hydroxynonenal; and HOE, 4-hydroxyoctenal.

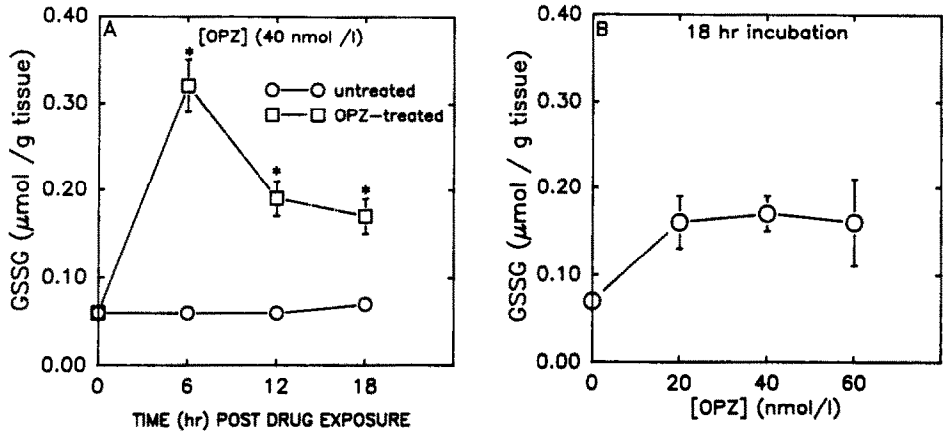

Fig. 1. Time (A) and drug concentration (B) related increases in GSSG levels in adult *S. mansoni* exposed to OPZ *in vitro*. GSSG was assayed as described under Materials and Methods in supernatant fractions (15,000 *g*, 20 min, 4°) prepared in 20 vol. 1% picric acid from worms incubated (at 37°) in HBSS (pH 7.4), in the presence of DMF (untreated controls) or OPZ. The data points are means ± SE of 3–4 independent determinations. Key: (*) $P < 0.01$, untreated vs treated worms.

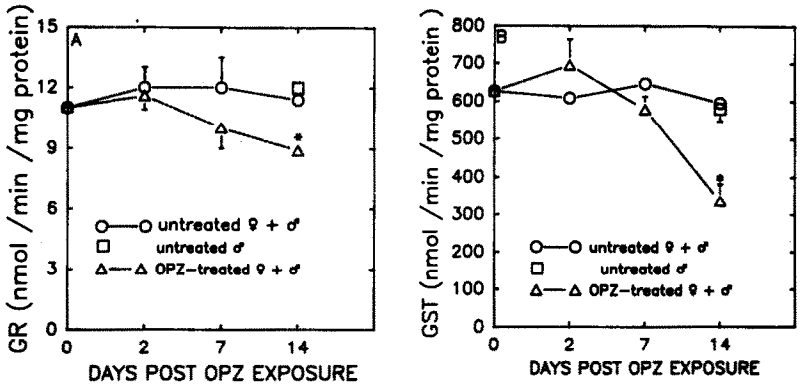

Fig. 2. Alterations in the activities of the enzymes GR (A) and GST (B) in adult *S. mansoni* exposed to OPZ *in vivo*, in mice. OPZ (100 mg/kg body wt) in a glycerol-cremophor El solution was administered orally to schistosome-infected mice. The untreated mice received the drug vehicle only. The worms were recovered by perfusion of the mesenteric system, and the enzymes were assayed, as described under Materials and Methods, in the cytosolic fractions of homogenates prepared in 20 vol. 100 mM Tris–HCl buffer, pH 7.4, + 150 mM KCl from the worms. The data points are means ± SE of 3–5 independent determinations, degrees of freedom = 7. Key: (*) $P < 0.01$, untreated vs treated.

In the present study we observed that, *in vitro*, GSH depletion in OPZ-treated adult *S. mansoni* led to increased GSSG formation and lipid peroxide levels; and long-term effects of the drug *in vivo*, in mice, resulted in significant decreases in the activities of the parasite GR and GST.

MATERIALS AND METHODS

Parasite material. S. mansoni (Puerto Rican strain) was used for these studies and was maintained through the snail *Biomphalaria glabrata* (Puerto Rican strain) and CD-1 mice (Charles River, St. Constance, Quebec, Canada). Mice received 150–200 cercariae percutaneously through shaved abdominal skin, essentially as described by Smithers and Terry [19]. Six to seven weeks following cercarial exposure, the mice were either killed and the adult worms harvested for *in vitro* studies, or they were used to study the effects of OPZ on the worms *in vivo*. Mice were killed by cervical dislocation, and the worms were recovered by perfusion of the mesenteric system with citrated saline (150 mM NaCl, 25 mM sodium-citrate) [19].

Reagents. All chemicals used were of analytical grade. 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), GSSG, cumene hydroperoxide (cumene-OOH), 1-chloro-2,4-dinitrobenzene (CDNB), *N,N*-dimethylformamide (DMF), thiobarbituric acid (TBA), trichloroacetic acid (TCA), bovine serum albumin (BSA) and cremophor El were obtained from the Sigma Chemical Co. (St Louis, MO). NADPH, GSH, and GR came from

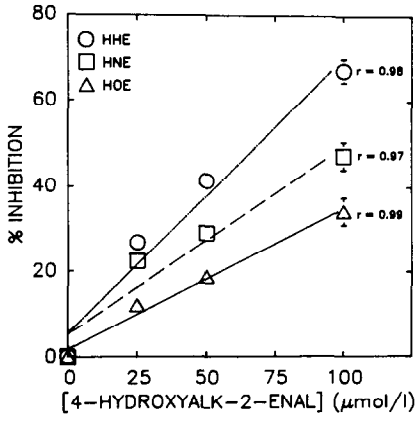

Fig. 3. Inhibition of the GST assay reaction by the 4-hydroxyalkenals HHE, HNE and HOE. The GST reaction mixture (in a 1-ml vol.) consisted of 100 mM potassium-phosphate buffer (pH 6.5), 1 mM GSH, 1 mM CDNB, 50 μl schistosome cytosolic fraction plus or minus one of the 4-hydroxyalkenals at the concentrations shown. Data points are means ± SE of 3 independent determinations. GST specific activity in the absence of the 4-hydroxyalkenals was 626 ± 17 nmol/min/mg protein.

Boehringer Mannheim, (Montréal, Canada); H_2O_2 was from Fisher Scientific (Montréal, Canada); 2-vinylpyridine (2-VP) from the Aldrich Chemical Co., (Milwaukee, WI); and Hanks' balanced salt solution (HBSS) from GIBCO (Burlington, Canada). OPZ was obtained from Rhône-Poulenc (Vitry, France), while the 4-hydroxyalk-2-enals, 4-hydroxyhexenal (HHE), 4-hydroxynonenal (HNE) and 4-hydroxyoctenal (HOE), were gifts from Dr H. Esterbauer, Institut für Biochemie, Universität Graz, Graz, Austria.

Exposure of worms to OPZ. The worms recovered from schistosome-infected mice were washed four times with, and subsequently incubated at 37° in, HBSS (pH 7.4) containing 5.5 mM glucose, 20 mM HEPES, 100 I.U. penicillin and 100 μg streptomycin/ml. OPZ was dissolved in DMF and added to give final concentrations of 20, 40 and 60 nM in the incubation medium. The maximum concentration of DMF used did not exceed 1%. The worms (five pairs in 2.5 ml) were incubated in 24-well micro-culture plates (Nunclon), at 37° with or without the drug, for up to 18 hr. At 6, 12 and 18 hr following incubation they were removed, washed twice in 150 mM NaCl solution, and prepared for biochemical assays. To study the effects on the parasite *in vivo,* mice with a 6 to 7-week-old *S. mansoni* infection were treated with 100 mg OPZ/kg body weight, administered by oral intubation. The drug was made up as a suspension in an aqueous solution of 25% (v/v) glycerol and 1% (v/v) cremophor El. The worms were recovered by perfusion, before drug-treatment (day 0), and 2, 7 and 14 days post-drug administration. They were counted, washed with HBSS, and prepared for biochemical studies.

Glutathione assay. To determine the effects of OPZ on the glutathione redox state, worms were homogenized in 20 vol. (w/v) 1% (v/v) picric acid in an ice-cooled glass homogenizer equipped with a Teflon pestle (Wheaton Scientific, Millville, NJ, U.S.A.), followed by centrifugation (15,000 *g*, at 4°, for 20 min). Glutathione was determined in the supernatant fraction by the enzymatic recycling assay in which glutathione in the sample is sequentially oxidized by DTNB and reduced by NADPH in a reaction catalyzed by GR [20, 21]. To determine GSSG the samples were incubated with 2-VP at 25° for 60 min before conducting the assay [21]. Glutathione concentrations were evaluated from a GSH standard curve, and are expressed in micromoles of GSH equivalents per gram of worm tissue [20, 21].

Lipid peroxidation assay. Lipid peroxide levels were determined in crude homogenates of OPZ-treated worms by the TBA assay, as described by Buege and Aust [22]. The assay reagent consisted of 15% (w/v) TCA, 0.38% (w/v) TBA and 0.25 N HCl. The worms were homogenized in 20 vol. (w/v) of cold 150 mM KCl, and 0.5 ml of the homogenate was mixed with 2.5 ml of the assay reagent. The mixture was heated for 15 min in a boiling water bath, cooled, and then centrifuged (1000 *g* for 10 min). The absorbance of the clear supernatant fraction was read at 535 nm against a reagent blank, and the concentration of the TBA-reactive substances was determined using extinction coefficient = 1.56×10^5/M/cm.

Enzyme assays. Worms incubated for 18 hr with or without 40 nM OPZ, or worms recovered from mice before OPZ treatment (day 0) and 2, 7, and 14 days post-treatment of mice with 100 mg/kg body weight were homogenized in 20 vol. (w/v) 100 mM Tris–HCl buffer, pH 7.4, containing 150 mM KCl. The activities of the enzymes GPO, GR and GST were assayed in the cytosolic fraction obtained after centrifugation of the homogenate at 100,000 *g*, at 4°, for 60 min. GPO (EC 1.11.1.9) was determined by the method which utilizes the NADPH-coupled reaction in which the GSSG resulting from the enzymatic oxidation of GSH is reduced by the NADPH-dependent GR [23]. The buffer consisted of 100 mM sodium-phosphate (pH 7.0) containing 5 mM EDTA, and the reaction was initiated with 2 mM H_2O_2 or 1.5 mM cumene-OOH. NADPH oxidation was followed at 340 nm (extinction coefficient = 6.2×10^3/mM/cm), at 25°. One unit of activity was defined as the oxidation of 1 μmol NADPH/min. GR (EC 1.6.4.2) was assayed according to Goldberg and Spooner [24]. The reaction mixture consisted of 120 mM potassium-phosphate buffer, pH 7.2 (37°), 15 mM EDTA, 65.3 mM GSSG and 9.6 mM NADPH. NADPH oxidation was followed at 340 nm (37°). One unit of activity was defined as the oxidation of 1 μmol NADPH/min. GST (EC 2.5.1.18) was determined as described by Habig *et al.* [25]. The assay mixture (in 1 ml) consisted of 100 mM potassium-phosphate buffer, pH 6.5, 1 mM CDNB, 1 mM GSH, and 50 μl of sample, and the change in absorbance was monitored at 340 nm, at 25°. One unit of activity was defined as the amount of enzyme catalyzing the formation of 1 μmol product/min. Since there was evidence of increased lipid peroxide levels in OPZ-treated schistosomes in the present study, and because 4-hydroxyalk-2-enals (aldehyde

Table 1. Drug concentration related decrease in total glutathione (GSH + GSSG) of adult *S. mansoni* exposed to OPZ *in vitro*

OPZ concn (nM)	Total schistosome glutathione (μmol GSH equiv./g tissue)	% Glutathione loss (of controls)
0	1.79 ± 0.21	0
20	1.43 ± 0.26	20.1
40	1.16 ± 0.11	35.2
60	0.94 ± 0.22*	47.5

Worms were incubated for 18 hr in HBSS (pH 7.4) at 37°, in the presence of the drug concentrations shown. The untreated controls were incubated with DMF, the drug solvent. Glutathione was determined as described under Materials and Methods in supernatant fractions of worm homogenates (in 20 vol., 1% picric acid) centrifuged at 15,000 *g* (20 min, 4°). The values are means ± SE of 3–4 independent experiments.

* Significantly different from untreated controls, $P < 0.05$.

products of lipid peroxidation) have been shown to be substrates for GST [26], the effects of aqueous solutions of HHE, HNE and HOE, on the GST reaction were assessed by including them in the assay mixture. The stock solutions of the 4-hydroxyalk-2-enals were kept in chloroform. To prepare the aqueous solutions, a fraction of the chloroform solution was evaporated at room temperature to almost dryness and the residue was dissolved in three times the volume of distilled water. The solution was vortexed for 1–2 min, and the traces of chloroform were removed by further evaporation. To estimate the concentration, the solution was diluted 1:1000 and the absorbance was measured at 223 nm (extinction coefficient = 1.375×10^4/M/cm).

Protein determination. Protein was determined by the method of Lowry *et al.* [27] using BSA as the standard.

Statistical analysis. The data were analyzed for statistical significance using the analysis of variance (ANOVA) test or Student's *t*-test. P values less than 0.05 were considered significant.

Table 2. GSH:GSSG ratios of OPZ-treated *S. mansoni in vitro*

Time post-incubation (hr)	OPZ (nM)	GSH:GSSG ratio
6	0	29
	40	5
12	0	27
	40	8
18	0	27
	20	8
	40	6
	60	5

The ratios were based on GSH and GSSG values determined as described under Materials and Methods, in 15,000 *g* (20 min, 4°) supernatant fractions of homogenates prepared in 20 vol. 1% picric acid from worms incubated (37°) in HBSS (pH 7.4) in the presence or absence of OPZ. The untreated worms were incubated with DMF, the drug solvent. Results are from 3–4 independent experiments.

RESULTS

Glutathione redox state in OPZ-treated schistosomes, in vitro. The GSH and GSSG levels in freshly isolated paired adult *S. mansoni* were 2.15 ± 0.14 (mean ± SE) and 0.06 ± 0.003 μmol/g worm tissue respectively. During the first 6 hr *in vitro*, the schistosome GSH levels declined by 27% from initial levels. However, the levels did not change significantly between 6 and 18 hr of incubation.

GSH levels of OPZ-treated (40 nM) worms, on the other hand, gradually but steadily declined, with time. By 18 hr of incubation, while the levels in the corresponding untreated controls were 1.73 ± 0.20 μmol/g worm tissue, the levels in the drug-treated worms had dropped to 1.00 ± 0.10 μmol/g tissue (i.e. to 58% of the corresponding controls), $P < 0.05$. There was also an apparent drug-concentration related decline in the schistosome GSH levels so that in worms incubated for 18 hr at concentrations of 20, 40 and 60 nM OPZ the levels dropped, respectively, to 73, 58 and 45% of the corresponding controls. Also observed, was a drug concentration related loss in the total glutathione levels in the treated worms (Table 1). The loss increased with increased drug concentration.

Concurrently, the GSSG levels in drug-exposed worms rose more than five-times above those of the corresponding controls, 6 hr following incubation, $P < 0.01$ (Fig. 1A). Although these levels remained significantly higher than those of the corresponding untreated controls, the levels declined significantly, from the peak of 0.32 μmol/g tissue, at 6 hr, to 0.14 μmol/g tissue by 18 hr. GSSG levels of the control worms, on the other hand, were not altered significantly, with time. While the GSSG levels measured in OPZ-treated worms 18 hr post-incubation were more than twice those of the untreated controls ($P < 0.01$), the levels were not altered significantly with increased drug concentration (Fig. 1B). The GSH:GSSG ratios in OPZ-treated worms dropped significantly, from between 27–29 to less than 8, 6–18 hr after exposure to 40 nM OPZ (Table 2). Up to 1% DMF (final concentration) did not alter the parasite glutathione redox state.

An examination of the activities of the schistosome GPO, GR and GST in worms incubated for 18 hr with 40 nM OPZ showed a significant increase in the activities of GPO towards cumene-OOH and GST with CDNB, whereas the activities of GPO towards

Table 3. Specific activities of the enzymes GPO, GR and GST in adult *S. mansoni* following *in vitro* exposure to OPZ

	Specific activity (nmol/min/mg protein)	
	Untreated	Treated
GPO (H_2O_2)	11.3 ± 0.3	10.9 ± 0.5
GPO (cumene-OOH)	26.7 ± 1.7	32.6 ± 1.8*
GR	11.2 ± 0.5	10.3 ± 0.3
GST	454.8 ± 26	526.5 ± 16*

The enzyme activities were assayed as described under Materials and Methods in the cytosolic fractions of homogenates prepared in 20 vol. 100 mM Tris–HCl buffer, pH 7.4, + 150 mM KCl from worms incubated for 18 hr (at 37°) in HBSS (pH 7.4) in the presence or absence (untreated) of 40 nM OPZ. The untreated controls were incubated with DMF, the drug solvent. The values are means ± SE of 4–5 independent determinations, degrees of freedom = 7.

* $P < 0.05$.

Table 4. Levels of TBA-reactive substances in adult *S. mansoni* exposed to OPZ *in vitro*

Time post-incubation (hr)	OPZ concn (nM)	TBA-reactive substances (nmol/g tissue)
6	0	43.7 ± 3.0
	40	55.3 ± 3.0
12	0	42.8 ± 3.1
	40	59.2 ± 3.4*
18	0	46.3 ± 0.6
	20	59.2 ± 3.5*
	40	71.3 ± 1.4*

The TBA-reactive substances were assayed as described under Materials and Methods, in homogenates prepared in 20 vol. 150 nM KCl from worms incubated (37°) in HBSS (pH 7.4) in the presence of DMF (untreated controls) or OPZ. Values are means ± SE of 4 independent determinations.

* $P < 0.05$, untreated vs treated.

H_2O_2, and of GR were not altered significantly during this period (Table 3).

Lipid peroxide levels in OPZ-exposed schistosomes. Following exposure of the worms to OPZ, lipid peroxide levels increased (as indicated by increased TBA-reactive substances). The levels in worms exposed to 40 nM OPZ increased above those of the control worms 6 hr post-incubation with the drug, and they were significantly higher than in the controls by 12 and 18 hr post-incubation (Table 4). Peroxide levels measured 18 hr post-incubation, in worms treated with 0, 20, and 40 nM OPZ, showed a drug-concentration related increase.

Antischistosomal activity of OPZ in vivo *effect on parasite GR and GST.* The proportion of the worms recovered by perfusion from infected mice, treated with 100 mg OPZ/kg body weight, gradually declined, over a 14-day period, to about 30% that of the controls given the glycerol-cremophor El solution alone. About 80% of these worms were males, giving a male : female ratio of 3.9, while the ratio in the worms from untreated mice was 1.2.

In worms exposed to OPZ *in vivo,* the GR activity gradually declined and by day 14 post-drug administration, the values (8.88 ± 0.05 nmol/min/mg protein) were significantly lower than those of the worms from control mice (11.40 ± 0.38 nmol/min/mg protein), $P < 0.01$ (Fig. 2A). A slight increase in the GST activity (from 608 ± 1.8 to 695 ± 6.4 nmol/min/mg protein) of drug-exposed worms, 2 days after drug administration to infected mice, was followed by a rapid decline ($P < 0.01$) in the enzyme activity to 335.7 ± 4.8 nmol/min/mg protein, while the activity in the corresponding controls (not exposed to the drug) was 595.6 ± 2.5 nmol/min/mg protein, by day 14 (Fig. 2B). The activities of both enzymes in the male worms not exposed to the drug were not significantly different from those of untreated paired worms.

Although the schistosome GPO activity, with cumene-OOH as a substrate increased slightly (from 26.5 ± 2.5 nmol/min/mg protein in the controls, to 31.2 ± 2.1 nmol/min/mg in the treated worms) 2 days after treatment of mice, the values were similar to those of the control worms by day 7, and did not alter by day 14. The GPO activity with H_2O_2, declined progressively with time but the decline was not significant. By day 14 following drug administration, the activity in the treated parasites (9.4 ± 1.7 nmol/min/mg protein) was not significantly different from that of the untreated worms (11.2 ± 0.1 nmol/min/mg protein).

The schistosome GST assay reaction was inhibited in a concentration related pattern, by the 4-hydroxy-alk-2-enals HHE, HNE and HOE, established products of lipid peroxidation (Fig. 3). At the lowest concentration used (25 μM), HHE inhibited the reaction by 27% (of the controls), HNE by 22% and HOE by 12%. At the highest substance concentration used (100 μM), the inhibition of the reaction was 67, 47 and 34% with HHE, HNE and HOE respectively.

DISCUSSION

The decline in schistosome GSH levels following OPZ exposure *in vitro* was associated with a dramatic increase in GSSG formation, hence a significant decrease in GSH : GSSG ratio. In addition, the total glutathione levels of the drug-treated parasites appeared to diminish. These observations suggest that increased GSH oxidation occurred in the OPZ-treated schistosomes, *in vitro*. However, the decline in GSSG levels observed following 6 hr of drug exposure (Fig. 1A) suggests loss in GSSG, possibly through efflux, and may partly account for the decrease in the total glutathione observed. A number of compounds, for instance the quinone menadione, have been reported to induce GSH oxidation [14, 16] in isolated rat hepatocytes, and the GSSG formed may be released from the cells by efflux [14]. Compounds such as menadione are known to undergo redox cycling with the generation of oxygen-free radicals [28]. The free radicals generated may oxidize

membrane lipids to initiate lipid peroxidation [10]. We do not know yet if OPZ undergoes redox cycling in schistosomes, but the levels of TBA-reactive substances (indicators of lipid peroxidation) were elevated in the worms treated with OPZ *in vitro,* above those in the untreated controls. The observed elevation in the TBA-reactive substances is perhaps particularly significant because these toxic oxidation products are usually rapidly neutralized by intracellular antioxidant systems such as glutathione. Some of the products generated during lipid peroxidation include peroxides and aldehydes [10]. The possible involvement of glutathione in the metabolism of such products is indicated by the initial increase in GPO (cumene-OOH) and GST activities in the schistosomes incubated with OPZ *in vitro* which catalyze reactions leading to the removal of peroxides and aldehydic products of lipid peroxidation [9, 12, 29]. Such processes may have contributed to the decrease in GSH levels through oxidation and/or conjugation reactions [9]. Whether the initial *in vitro* and *in vivo* increases in the activities of GPO and GST are due to an increase in enzyme synthesis or an increase in rate based possibly on isoenzyme changes, will require further investigation.

Although previous studies [2, 6, 7], and the present study, seem to indicate that the OPZ effects result in perturbations of schistosome glutathione metabolism, it is still not clear how the drug exerts its toxicity to the parasite. Frappier *et al.* [7] observed that schistosomes isolated from infected mice 6 hr following OPZ (500 mg/kg) administration, died 48–72 hr later, under *in vitro* conditions, whereas worms from untreated mice survived for several days, under similar conditions. In a previous study we observed a significant mortality in adult *S. mansoni* incubated *in vitro* for 18 hr with OPZ (40 nM) or other GSH-depleting agents (CDNB, buthionine sulfoximine), in the presence of glucose–glucose oxidase, a H_2O_2-generating system [30]. We also have some evidence to indicate that oxygen metabolites generated by peritoneal exudate cells from schistosome- or Bacillus Calmette-Guerin (BCG)-infected mice may kill OPZ-treated adult schistosomes.* Accumulation of GSSG [9] or products of lipid peroxidation [10] may produce toxic effects. It is likely that the elevated levels of GSSG and products of lipid peroxidation in the OPZ-treated worms could produce toxic effects in the parasite. The presence of H_2O_2 could be expected to exacerbate these effects and enhance killing of the parasites. Reactive oxygen species may be generated by activated host phagocytic cells [31–33]. It is therefore possible that OPZ acts synergistically with host cell generated oxygen metabolites to kill the parasites, *in vivo.*

Under *in vivo* conditions, significant decreases in the activities of schistosome GR and GST were observed 14 days following drug administration to infected mice. This drug associated decrease in the parasite glutathione enzymes coincided with a significant decrease in the number of worms, particularly the females, recovered by perfusion from the drug-treated mice. It is unlikely that the absence of the female worms contributed to the decline in the activities of the enzymes because the enzyme activities in a male population not exposed to OPZ were still significantly higher than those of the drug-exposed worms. The fact that these effects occurred several days after exposure of the worms to the drug is consistent with the fact that OPZ is a slow-acting drug and up to 2 months are required for full antischistosomal activity [2].

The effect of OPZ on the parasite GST activity may compromise the ability of the parasite to neutralize products of lipid peroxidation. We observed that the 4-hydroxyalk-2-enal aldehydes, known products of lipid peroxidation, inhibited schistosome GST reaction with CDNB as a substrate. Whether decreased GST activity observed in the drug-treated schistosomes, *in vivo*, was due to the presence of lipid peroxidation products requires further investigation. Schistosome GST is of further interest in view of the fact that it is being investigated as a potential vaccine molecule for protection against schistosomiasis [34, 35].

Although the magnitude of the changes in the enzyme activities was different between worms exposed to the drug *in vitro* and those exposed *in vivo*, the pattern of the changes was more or less similar, at least between 18 and 48 hr of drug exposure. For instance, the significant increase in the activity of GPO (cumene-OOH) *in vitro* is reflected *in vivo* by the apparent increase in the enzyme activity observed 2 days post-drug exposure of the worms. Similarly, the increase in GST activity at 18 hr *in vitro* is also seen *in vivo* 2 days after drug exposure, but to a smaller extent. Alterations in the activities of GPO (H_2O_2) or GR whether under *in vitro* or *in vivo* conditions were not obvious during the first 2 days.

In summary, OPZ may induce lipid peroxidation in adult *S. mansoni.* The parasite may initially attempt to detoxify some of the products generated during this process as indicated by increased GPO (cumene-OOH) and GST activities, and GSH depletion. However, as lipid peroxidation products accumulate, they may subsequently inhibit parasite GST activity. Other drug-induced changes such as decreased GR activity (and to a smaller extent GPO with H_2O_2) would further perturb parasite glutathione metabolism. Since the glutathione pathway may be important in the protection of organisms against oxidative damage or toxic substances such as those generated during lipid peroxidation, this series of events may increase the susceptibility of the parasite to damage by reduced oxygen species generated by activated host phagocytic cells, and could be linked with the antischistosomal action of the drug.

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REFERENCES

1. Leroy JP, Barreau M, Cotrel C, Jeanmart C, Messer M and Benazet F, Laboratory studies of 35 972 RP, a new schistosomicidal compound. In: *Current Chemotherapy, Proceedings of the 10th International Congress of Chemotherapy* (Eds. Siegenthaler W and Luthy R), Vol. I, pp. 148–150. American Society for Microbiology, Washington, DC, 1978.
2. Bueding E, Dolan P and Leroy JP, The antischistosomal activity of oltipraz. *Res Commun Chem Pathol Pharmacol* **37**: 293–303, 1982.
3. Woehrlé R, Sung RTM and Garin JP, One-day treatment of *Schistosoma mansoni* and *Schistosoma haematobium* infections with oltipraz (35 972 R.P.). In: *Current Chemotherapy and Infectious Diseases* (Eds. Nelson JD and Grassi C), pp. 1109–1111. American Society for Microbiology, Washington, DC, 1980.
4. Gentillini M, Duflo B, Richard-Lenoble D, Brucker G, Danis M, Niel G and Meunier Y, Assessment of 35 972 R.P. (oltipraz), a new antischistosomal drug against *Schistosoma haematobium, Schistosoma mansoni* and *Schistosoma intercalatum*. *Acta Trop (Basel)* **37**: 271–274, 1980.
5. Kardaman MW, Fenwick A, Igail ABE, Tayeb ME, Bennett JL and Daffalla AA, Field trials with oltipraz against *Schistosoma mansoni* in the Gezira Irrigated Area, Sudan. *J Trop Med Hyg* **88**: 95–100, 1985.
6. Morrison DD, Thompson DP, Semyn DR and Bennett JL, Acute effects of oltipraz on adult *Schistosoma mansoni* and its antagonism *in vitro*. *Biochem Pharmacol* **36**: 1169–1171, 1987.
7. Frappier F, Azoulay M and Leroy JP, Effect of oltipraz on the metabolism of glutathione in *Schistosoma mansoni*. *Biochem Pharmacol* **37**: 2864–2866, 1988.
8. Sturrock RF, Oltipraz withdrawn from clinical trials. Correspondence. *Trans R Soc Trop Med Hyg* **81**: 528, 1987.
9. Meister A and Anderson ME, Glutathione. *Annu Rev Biochem* **52**: 711–760, 1983.
10. Horton AA and Fairhurst S, Lipid peroxidation and mechanisms of toxicity. *CRC Crit Rev Toxicol* **18**: 27–79, 1987.
11. Ketterer B, Coles B and Meyer DJ, The role of glutathione in detoxication. *Environ Health Perspect* **49**: 59–69, 1983.
12. Mannervik B, The isoenzymes of glutathione transferase. *Adv Enzymol* **57**: 357–417, 1985.
13. Ketterer B, Detoxication reactions of glutathione and glutathione transferases. *Xenobiotica* **16**: 957–973, 1986.
14. Eklöw L, Thor H and Orrenius S, Formation and efflux of glutathione disulfide studied in isolated rat hepatocytes. *FEBS Lett* **127**: 125–128, 1981.
15. Brigelius R, Lenzen R and Sies H, Increase in hepatic mixed disulphide and glutathione disulphide levels elicited by paraquat. *Biochem Pharmacol* **31**: 1637–1641, 1982.
16. Di Monte D, Ross D, Bellomo G, Eklöw L and Orrenius S, Alterations in intracellular thiol homeostasis during the metabolism of menadione by isolated rat hepatocytes. *Arch Biochem Biophys* **235**: 334–342, 1984.
17. Younes M and Siegers CP, Lipid peroxidation as a consequence of glutathione depletion in rat and mouse liver. *Res Commun Chem Pathol Pharmacol* **27**: 119–128, 1980.
18. Siegers CP, Hubscher W and Younes M, Glutathione-*S*-transferase and GSH-peroxidase activities during the state of GSH-depletion leading to lipid peroxidation in rat liver. *Res Commun Chem Pathol Pharmacol* **37**: 163–169, 1982.
19. Smithers SR and Terry RJ, The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology* **55**: 695–700, 1965.
20. Tietze F, Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione: application to mammalian blood and other tissues. *Anal Biochem* **27**: 502–522, 1969.
21. Griffith OW, Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* **106**: 207–212, 1980.
22. Buege JA and Aust SD, Microsomal lipid peroxidation. In: *Methods in Enzymology* (Eds. Packer L and Fleischer S), Vol. 52, pp. 302–310. Academic Press, New York, 1978.
23. Paglia DE and Valentine WN, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* **70**: 158–169, 1967.
24. Goldberg DM and Spooner RJ, Glutathione reductase. In: *Methods in Enzymatic Analysis* (Ed. Bergmeyer HU), Vol. III, 3rd Edn, pp. 258–265. Verlag Chemie, Weinheim, 1983.
25. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
26. Ålin P, Danielson UH and Mannervick B, 4-Hydroxyalk-2-enals are substrates for glutathione transferase. *FEBS Lett* **179**: 267–270, 1985.
27. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
28. Wefers H and Sies H, Hepatic low-level chemiluminescence during redox cycling of menadione and the menadione-glutathione conjugate: relation to glutathione and NAD(P)H:quinone reductase (DT-diaphorase) activity. *Arch Biochem Biophys* **224**: 568–578, 1983.
29. Burk RF, Nishiki K, Lawrence RA and Chance B, Peroxide removal by selenium-dependent and selenium-independent glutathione peroxidases in hemoglobin-free perfused rat liver. *J Biol Chem* **253**: 43–46, 1978.
30. Mkoji GM, Smith JM and Prichard RK, Antioxidant systems in *Schistosoma mansoni*: evidence for their role in protection of the adult worms against oxidant killing. *Int J Parasitol* **18**: 667–673, 1988.
31. Klebanoff SJ, Oxygen metabolism and the toxic properties of phagocytes. *Ann Intern Med* **93**: 480–489, 1980.
32. Nathan CF, Secretion of oxygen intermediates: role in effector functions of activated macrophages. *Fed Proc* **41**: 2206–2211, 1982.
33. Johnston RB Jr and Kitagawa S, Molecular basis for the enhanced respiratory burst of activated macrophages. *Fed Proc* **44**: 2927–2932, 1985.
34. Smith DB, Davern KM, Board PG, Tiu WU, Garcia EG and Mitchell GF, M, 26,000 antigen of *Schistosoma japonicum* recognized by resistant WEHI 129/J mice is a parasite glutathione *S*-transferase. *Proc Natl Acad Sci USA* **83**: 8703–8707, 1986.
35. Smith DB, Rubira MR, Simpson RJ, Davern KM, Tiu WU, Board PG and Mitchell GF, Expression of an enzymatically active parasite molecule in *Escherichia coli: Schistosoma japonicum* glutathione *S*-transferase. *Mol Biochem Parasitol* **27**: 249–256, 1988.