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MECHANISMS OF INACTIVATION OF *SCHISTOSOMA MANSONI* AND MAMMALIAN GLUTATHIONE *S*-TRANSFERASE ACTIVITY BY THE ANTISCHISTOSOMAL DRUG OLTIPRAZ

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Abstract—Glutathione *S*-transferase (GST) purified from *Schistosoma mansoni* or human placenta was inhibited by the antischistosomal drug oltipraz (OPZ) in a time- and concentration-dependent manner. Inhibition of placenta GST was complete at a low concentration of drug, whereas that of parasite GST was incomplete and relatively high amounts of OPZ were needed to reach 50% inhibition. Complete reactivation of GST from placenta was achieved with dithiothreitol (DTT) and other sulfhydryl-containing compounds, while the inactivation of parasite GST was irreversible. The oxy-derivative of OPZ (RP 36 642), in which the thione sulfur is replaced with oxygen, did not inhibit GST activity. There were no differences between OPZ and RP 36 642 in their patterns of binding to the hydrophobic non-substrate site of GST. GST from the placenta incorporated much higher levels of [^{14}C]*N*-ethylmaleimide compared to schistosome GST. The incorporation of [^{14}C]*N*-ethylmaleimide by GST was inhibited by OPZ but not by RP 36 642. Yeast and *S. mansoni* hexokinases were similarly inhibited by OPZ but not by RP 36 642. Both hexokinase preparations recovered their activity following incubation with DTT. These data suggest that the inactivation of these enzymes by OPZ is a result of its interaction with their SH groups. Thus, the antischistosomal activity of OPZ may be accounted for by its interaction with the SH groups of macromolecules in general.

Oltipraz [4-methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione] (OPZ†) is a thionosulfur compound that has very good antischistosomal activity [1]. The efficacy of OPZ against schistosomes has been evaluated in numerous clinical trials [2–6], and the drug is active against all the major species of schistosomes that infect humans. The action of OPZ on parasites is slow and sometimes up to 2 months are required for the realization of the full antischistosomal activity [7]. The efficacy of OPZ also varies with the species of animal studied, being better in monkeys (20 mg/kg) than in mice (50 mg/kg) [1]. The efficacy of OPZ is reduced in animals pretreated with inducers of drug metabolising enzymes such as phenobarbital or butyl-hydroxyanisole [7]. Thus, metabolism and disposition of OPZ are important factors in its efficacy. Approximately thirteen different metabolites of OPZ were isolated and identified after metabolism in the mouse [8]. However, it is still not clear whether the parent compound or the metabolites are responsible for the antiparasitic activity of this drug. Like many other antischistosomal agents, the mechanism of action of OPZ is poorly understood.

OPZ causes the classical hepatic shift of the schistosomes from the mesenteries to the liver [7]. One of the earliest biochemical effects in the worms within the first hour after exposure to OPZ *in vitro* is the lowering of reduced glutathione (GSH) levels [9], and this precedes hepatic shift of the worms. It is believed that OPZ exerts its antiparasitic effects by interfering with glutathione metabolism in the parasite [7, 9]. This may cause metabolic disturbances in various key areas and possibly the elimination of the parasite by the host immune mechanisms, possibly by reducing defences against reactive oxygen intermediates [10]. Analogues of OPZ that lack schistosomicidal activity do not affect parasite glutathione levels [7].

Recently, OPZ was shown to decrease the activities of the enzymes glutathione reductase (GR) [10, 11] and glutathione *S*-transferase (GST) [10] in addition to depleting glutathione. One consequence of this was the increased susceptibility of the adult schistosomes to killing by immune cells [12]. *In vitro* OPZ does not inhibit GR activity but some of its metabolites known to be produced *in vivo* [8] are inhibitory [11]. These metabolites exhibit some antischistosomal action *in vitro* provided they are derived from OPZ or its active analogues. It was therefore suggested that OPZ is a pro-drug [11, 13], and the depletion of GSH levels was attributed to the inhibition of GR following metabolism of OPZ and hence the reduced capacity to convert oxidized

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† Abbreviations: OPZ, oltipraz; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; GR, glutathione reductase; CDNB, 1-chloro-2,4-dinitrobenzene; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, dithiothreitol; SH, sulfhydryl; NEM, *N*-ethylmaleimide; DMF, *N*,*N*-dimethylformamide; and ANS, 8-anilinonaphthalene-1-sulfonate.

glutathione (GSSG) to GSH. However, there is no evidence that schistosomes metabolize OPZ, and the metabolites (disulfides) that inhibit GR activity *in vitro* have no antischistosomal action *in vivo* [13].

Previously, we demonstrated that OPZ inhibits schistosome GST activity *in vivo* in a non-isoenzyme selective manner [14]. Our subsequent *in vitro* studies showed that purified schistosome GST is inhibited directly by OPZ in a time- and concentration-dependent manner [15]. The inhibition of GST from *Schistosoma mansoni* by OPZ was essentially an irreversible process. The oxy-analogue of OPZ, RP 36 642, had no effect on GST activity. However, the mechanisms of GST inactivation by OPZ were not investigated and comparisons between parasite and mammalian (host) enzymes were not made. Also, it was not clear whether the mechanisms by which GST was inhibited by OPZ were specific for that enzyme alone or extend to other proteins.

GSTs are ubiquitous, dimeric, multifunctional cytosolic proteins that occur as isoenzymes and they interact with several structurally dissimilar electrophilic substrates via the catalytic site to promote conjugation to GSH, a detoxification reaction. In addition, GST isoenzymes bind to numerous hydrophobic compounds including some clinically available anthelmintics [16] and antibiotics [17], facilitating their solubility and intracellular transport [18]. This is also thought to be a passive detoxification mechanism [16]. Kinetic studies suggest that there are separate binding sites for GSH (G-site), an electrophilic site (H-site) and a non-substrate hydrophobic site [19, 20]. Although the substrate and non-substrate sites are independent [20], the binding of hydrophobic ligands can interfere with the catalytic activity of GST. Thus, OPZ may interact with any of these sites to inhibit the activity of GST. However, previous data suggest that OPZ is not a substrate for GST [15] and hence does not compete with the substrates. It is not clear if OPZ binds to the non-substrate site of GST to inhibit the enzyme activity. Inactivation of enzyme activity could also be accomplished via binding of the drug to reactive amino acid residues, particularly the sulfhydryls (SH groups) of GST that may be important for catalytic functions. SH group modification has been shown to be a mechanism of inactivation for numerous enzymes whose activities are redox controlled via thiol–disulfide exchanges [21]. The reaction of OPZ with SH groups of macromolecules could result in a covalently bound drug complex, and if such macromolecules are essential enzymes, the reaction could result in slow death of the parasite; OPZ is a slow-acting drug. The present study is designed to provide some insights into the possible mechanisms of inactivation of schistosome GST by OPZ and to determine if such reactions apply to other enzymes.

MATERIALS AND METHODS

Chemicals. OPZ and its oxy-analogue (RP 36 642) were gifts from Rhone-Poulenc Sante, France. *N*-Ethylmaleimide (NEM), bovine serum albumin (BSA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), GSH, 8-anilinonaphthalene-1-sulfonate (ANS), bilirubin, hematin and GST from human placenta were purchased from the Sigma Chemical Co. (St Louis, MO). Yeast hexokinase was obtained from Boehringer Mannheim (Quebec, Canada). Radiolabelled *N*-ethylmaleimide ([^{14}C]NEM) was obtained from Amersham (Arlington Heights, IL). All other chemicals were of analytical grade.

GST preparation. Native GST from *S. mansoni*, recombinant GST isoenzymes (Sm 28 and Sm 26) produced according to the procedures of Smith *et al.* [22], and mouse liver GST were purified via a GSH-agarose affinity column [23]. GST activity was routinely determined with GSH and CDNB as substrates [24]. Enzyme preparations were concentrated with Amicon YM-10 filters. To remove GSH from enzyme preparations, the GST was passed through a column of Sephadex G-25 equilibrated with 0.1 M potassium phosphate buffer (pH 7.5) containing 0.5 mM EDTA and 15% (v/v) glycerol [25] followed by dialysis in the same buffer for 48 hr at 4°. Protein was determined by the method of Bradford [26].

Competitive fluorescence spectroscopy. Binding studies were performed with a Perkin–Elmer recording fluorometer according to the procedures of Ketly *et al.* [25]. The assays were carried out at room temperature in a total volume of 3 mL. The assay mixture contained 5 μM ANS, 1 mM GSH, 0.1 M KPO_4 buffer (pH 7.5), 0.2 mM EDTA and approximately 0.1 μM GST protein (assuming a molecular weight of 50,000 daltons). The excitation filter was set at 365 nm and emission at 495 nm. The assay mixture was titrated with a concentrated test ligand in *N,N*-dimethylformamide (DMF), (OPZ, bilirubin, hematin or RP 36 642) in 1-μL aliquots up to a maximum volume of 10 μL. The solution was mixed after each addition and changes in relative fluorescence were monitored, with corrections for solvent effects.

GST activity assay. GST was incubated with the drugs under test, in 0.1 M Tris–HCl (pH 7.8) at 25° for 1 hr. Following preincubation, 50-μL aliquots of the mixture were removed and used to assay for GST activity with GSH and CDNB as substrates. In these reactions, initial velocities were always 0.05 absorbance units/min or less. Drugs were excluded from control incubations with a concentration of DMF not exceeding 1%. For reactivation, the inactivated GST preparations were incubated with up to 50 mM dithiothreitol (DTT) or other thiol compounds for 1 hr, and activity was measured with CDNB and GSH as substrates [25].

Hexokinase activity assay. The enzyme activity was determined spectrophotometrically at 25° in Tris–HCl buffer (pH 7.8) in a system coupled with glucose-6-phosphate dehydrogenase [27]. Initial rate measurements were done by following the reduction of $NADP^+$ at 340 nm. Control incubations were such that the velocities were 0.05 absorbance units/min or less. The inhibitory effects of OPZ and of RP 36 642 were assessed by preincubating a hexokinase suspension in the presence of a 500 μM concentration of these compounds for 1 hr at 25°. Purified yeast hexokinase as well as *S. mansoni* cytosolic fractions were used as sources of enzyme in the various assays.

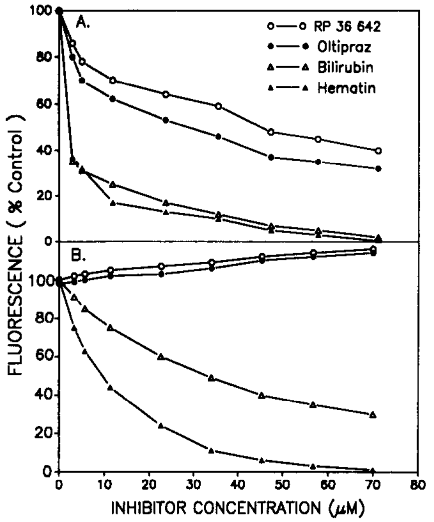

Fig. 1. Effects of different test ligands (OPZ, RP 36 642, bilirubin and hematin) on the fluorescence of GSTs. The assay was carried out as described in Materials and Methods, using 0.1 μM GST from (A) mouse liver and (B) *S. mansoni*. Results are expressed as percent of control (DMF without test ligands). The starting absorbance (control) was set at approximately 300 units.

For reactivation of inhibited hexokinase, the enzyme solution was incubated for another hour in the presence of up to 50 mM DTT; enzyme activity was then determined. The activities are given as percent of control activity (without inhibitors).

Binding of [^{14}C]*NEM to protein SH groups.* GSH-free GST, yeast hexokinase or trichloroacetic acid (TCA)-precipitable cytosolic fractions from *S. mansoni* were incubated with 10 μmol of [^{14}C]NEM (122,800 cpm) in 1 mL of 0.1 M Tris–HCl (pH 7.8) at 25° for 1 hr. Aliquots (300 μL) were added to 3 mL of 10% TCA followed by the addition of 100 μL BSA (10 mg/mL) [28]. Precipitated proteins were collected by centrifugation at 300 rpm for 5 min. The supernatant was discarded and the precipitated proteins were dissolved in 0.7 mL of 0.1 N NaOH. This procedure was repeated twice to remove free [^{14}C]NEM. Precipitated and solubilized samples were mixed with 4 mL of scintillation fluid and protein radioactivity was determined. For the inhibition of [^{14}C]NEM binding to proteins, the samples were preincubated with OPZ, RP 36 642, DTNB or bilirubin for 1 hr before the addition of [^{14}C]NEM. DTNB (Ellman's reagent) reacts with SH groups while bilirubin binds to the non-substrate hydrophobic site of GST and these were used as additional controls.

RESULTS

GSH-free GST was utilized to assess the binding of ANS to the non-substrate ligand site of the enzyme. In the absence of protein, no fluorescence was observed and GSH enhanced ANS fluorescence, but proved to be inhibitory at higher concentrations. In all subsequent assays, 1 mM GSH was used with 5 μM ANS in order to assess the possibility that OPZ inhibits GST by binding to the hydrophobic non-substrate site of the enzyme. DMF, the drug solvent, had no effect on fluorescence intensity. Results in Fig. 1A demonstrate the inhibitory effects of test ligands on ANS fluorescence in the presence of GST purified from livers of female CD-1 mice. Hematin and bilirubin are known to bind to the non-substrate hydrophobic site of GST [25]. The highest fluorescence quenching was obtained with hematin, closely followed by bilirubin. RP 36 642 and OPZ also quenched ANS fluorescence, although to a lesser extent. All these compounds therefore interact with the non-substrate hydrophobic binding site of mouse liver GST. However, only hematin and bilirubin reduced fluorescence of ANS bound to native schistosome GST, whereas OPZ and RP 36 642 did not (Fig. 1B). The differences between hematin and bilirubin are much more pronounced with schistosome GST than with the mammalian enzyme. Because the Sm 26 (26 kDa GST isoenzyme) constitutes only 5% of the native GST from *S. mansoni* [29], recombinant isoenzymes were used. Both Sm 28 and Sm 26 did not appear to interact with OPZ via the hydrophobic site (data not shown). It is therefore unlikely that OPZ inhibits schistosome GST by interacting with its hydrophobic site.

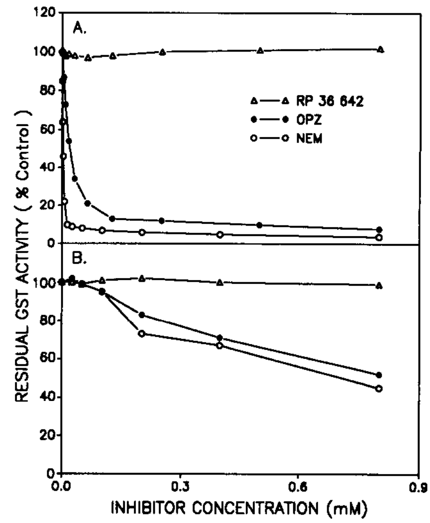

Fig. 2. Inhibition of GST activity by OPZ, RP 36 642 and NEM. GST from (A) human placenta and (B) *S. mansoni* was incubated with different concentrations of inhibitors in Tris–HCl buffer (pH 7.8) at 25° for 1 hr. Enzyme samples (50 μL) were transferred into a cuvette containing 1 mM GSH and 1 mM CDNB, and conjugation was measured at 340 nm. Experiments were carried out in triplicate and results are expressed as percent of control (incubations with drug solvents only). In the control incubations, GST activity was 25.28 ± 3.45 μmol/min/mg protein for *S. mansoni* and 38.91 ± 5.72 μmol/min/mg protein for the sample from human placenta. These control values did not change significantly during the incubation period.

To assess the possible interaction of OPZ with

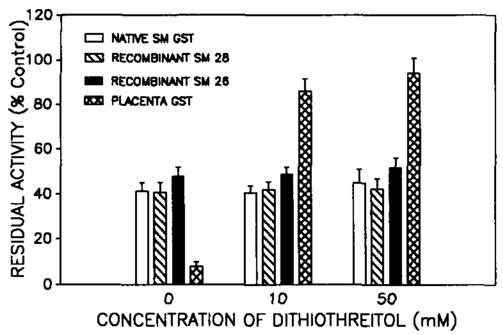

Fig. 3. Effects of dithiothreitol on the activity of OPZ-inactivated GST. Native and recombinant GST from *S. mansoni* and native GST from human placenta were incubated with 500 μM OPZ for 1 hr (inactivation) followed by a 1-hr incubation with DTT (reactivation). GST activity was measured before and after incubation with DTT. Control incubations did not contain OPZ and results are expressed as percent of control (± SEM) and are the means of three determinations. The absolute values for the control incubations were 28.89 ± 2.67, 20.43 ± 2.18, 12.55 ± 1.38 and 35.75 ± 6.02 μmol/min/mg protein for native *S. mansoni* GST, recombinant Sm 28, recombinant Sm 26 and GST from human placenta, respectively. These initial activities did not change significantly during the incubation period.

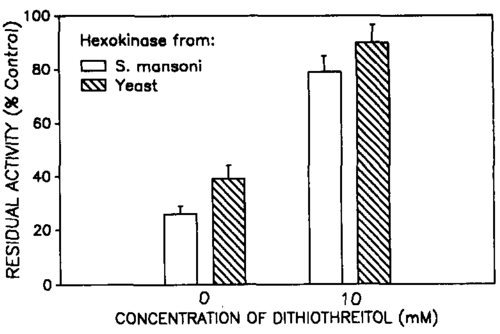

Fig. 4. Effects of OPZ on the activity of hexokinase from yeast and from *S. mansoni* cytosolic fractions. The enzyme preparations were incubated with 500 μM Tris–HCl (pH 7.8) for 1 hr at 25° (inactivation) and the activity was determined as described in Materials and Methods. The inactivated enzyme preparations were incubated with 10 mM DTT for 1 hr at 25° (reactivation) before enzyme determination. Assays were performed in triplicate and results are expressed as percent of control (± SEM) of at least three determinations. The initial control activity was 134.98 ± 9.05 and 18.47 ± 1.94 nmol/min/mg protein for yeast and *S. mansoni* derived hexokinases, respectively, and did not change significantly during the incubation period.

protein sulfhydryl groups, the inhibitory effects of NEM (which specifically alkylates SH groups) on GST were compared to those of RP 36 642 and OPZ. GST from human placenta was used in these assays because of its highly reactive SH groups that are known to be essential for activity [28, 30–32]. Results of this comparison are shown in Fig. 2. RP 36 642 had no effect on the activities of either mammalian or parasite GST. GST from the human placenta was extremely sensitive to both OPZ and NEM at low concentrations (Fig. 2A). GST from *S. mansoni* on the other hand (Fig. 2B) was inhibited by both OPZ and NEM at relatively high concentrations. Reactivation of GST from human placenta was achieved following incubation with dithiothreitol, whereas the parasite GST could not recover its activity in the presence of SH compounds (Fig. 3). The inhibition of both forms of GST by NEM was not reversible upon addition of thiols. Reactivation of the OPZ-inactivated placenta GST could also be accomplished by incubation with GSH, mercaptoethanol, L-cysteine or *N*-acetyl L-cysteine but not with GSSG (data not shown). To determine whether other non-GST SH-dependent enzymes are vulnerable to the effects of OPZ, yeast and *S. mansoni* hexokinase were incubated with the drug for 1 hr before determination of activity. OPZ inhibited both yeast and parasite hexokinase and the effects were reversed by incubation with DTT (Fig. 4). To further verify the involvement of sulfhydryl groups in GST and hexokinase inactivation by OPZ and NEM, [^{14}C]NEM was used to assess binding to purified enzymes. NEM bound to GST from human placenta *S. mansoni* as well as to TCA-precipitable soluble proteins from the parasite (Fig. 5). However, more [^{14}C]NEM was incorporated into mammalian than parasite GST. Preincubation of GST with OPZ or DTNB reduced the amount of [^{14}C]NEM incorporated into the enzyme (Fig. 6), whereas RP 36 642 and bilirubin had no effects on such binding. Figure 7 demonstrates that OPZ but not the oxy-analogue inhibited the binding of [^{14}C]NEM to hexokinase.

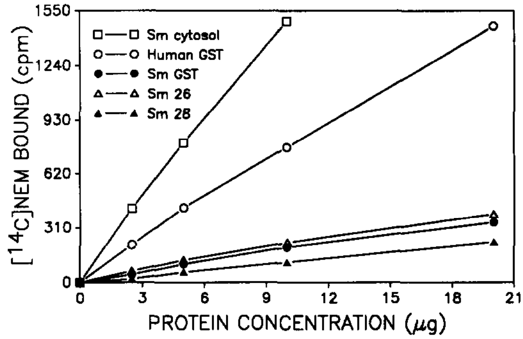

Fig. 5. [^{14}C]NEM labelling of *S. mansoni* cytosolic, proteins and GST. The reaction mixture contained different concentrations of protein and 10 μmol [^{14}C]NEM in Tris–HCl (pH 7.8); incubations were carried out for 1 hr at 25°. The procedure for determining the radioactivity of the proteins following specific incorporation of NEM is described in Materials and Methods.

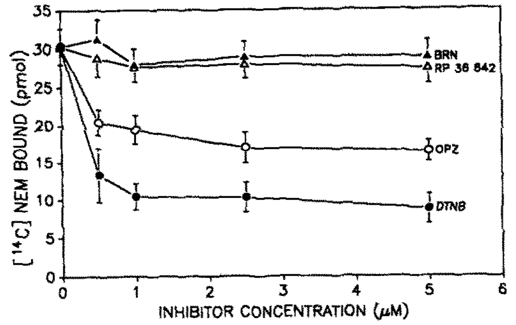

Fig. 6. Inhibition of NEM binding to GST from human placenta by OPZ. GST (10 μg) was preincubated with different concentrations of OPZ, RP 36 642, DTNB or bilirubin for 1 hr at 25° before the addition of 10 μmol [^{14}C]NEM. After incubation for another hour, bound [^{14}C] in the presence and in the absence of inhibitors was quantified by scintillation counting as described earlier. Results are shown as the amount of [^{14}C]NEM bound ± SEM of at least three determinations.

DISCUSSION

In the presence of GSH, GST enhanced the fluorescence of ANS dramatically. This indicates that ANS binds to mammalian and parasite GST via the hydrophobic non-substrate binding site. This system could be used to study the binding of other ligands to the same site by observing their ability to quench the fluorescence of this GST–ANS complex. Hematin and bilirubin are well known inhibitors of mammalian GST via their interaction with the hydrophobic site. This was confirmed in this study and used as control. OPZ and RP 36 642 were less effective in quenching the mammalian GST–ANS complex and had no effect on the fluorescence of the parasite GST–ANS complex. This suggests that there may be fundamental differences between parasite and host GST with respect to binding of hydrophobic xenobiotics. Sm 26 and Sm 28, products of different schistosome GST genes, had similar patterns of interaction with various ligands. This suggests similarities between the non-substrate binding capacities of the two major isoenzymes of *S. mansoni*. The lack of differences between RP 36 642 and OPZ in their ability to interact with both the mammalian and parasite GST hydrophobic site suggests that this mechanism may not account for the inhibitory activity on both parasite and mammalian GST. Also, the lack of differences implies that the binding of OPZ to GST via the hydrophobic site may not be an important determinant of antiparasitic action of this drug, because RP 36 642 has no antischistosomal activity [7]. From these experiments it appears reasonable to assume that the inhibitory effects of OPZ on GST activity are due to mechanisms other than binding to the hydrophobic site.

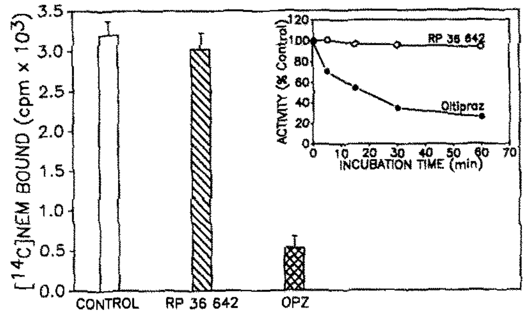

Fig. 7. Inhibition of NEM binding to yeast hexokinase by OPZ. Hexokinase (30 μg) was preincubated with 500 μM OPZ or RP 36 642 for 1 hr at 25° followed by the addition of radiolabelled NEM. After further incubation to allow incorporation of radioactivity, the samples were assessed by scintillation counting. Results are means ± SEM of at least three determinations. The insert shows the pattern of inhibition of yeast hexokinase activity by OPZ. The initial control activity was 134.97 ± 9.05 nmol/min/mg yeast protein and did not change significantly during incubation.

Alternatively, OPZ may interact with specific reactive amino acid residues, particularly the protein SH groups. NEM specifically alkylates free SH groups, and if these are essential for enzyme activity of the particular protein, inactivation or inhibition may result. The inhibitory activities of OPZ and NEM on parasite and mammalian GST were very similar, and OPZ inhibited the binding of [^{14}C]NEM to GST and other proteins. This suggests a possible common mechanism of interaction between OPZ and NEM, that is binding to SH groups. On the other hand, RP 36 642 had no effect on the activities of any of the GSTs investigated and did not inhibit the binding of NEM to proteins. Bilirubin binding to the non-substrate hydrophobic site did not seem to interfere with NEM binding to SH groups. GST from human placenta is predominantly of the class π (80%) and this class of GST from various mammalian tissues has 4 cysteines at corresponding positions (14, 47, 101 and 169th) [28]. Modification of the highly reactive 47th cysteine alone is sufficient to account for the extreme sensitivity of this form of GST to NEM [28, 30], hydrogen peroxide (H_2O_2) [31] and biological disulfides [32]. The inactivation of GST with H_2O_2 and biological disulfides is reversible with mammalian enzymes, upon addition of reducing agents like dithiothreitol. Specifically, it was determined that 1 mole of NEM reacts with 1 mole of GST π [28]. Using an NEM spin label [30], it was confirmed that there are two reacting thiols per GST π dimer. Titration of GST SH groups before and after addition of NEM also supports the reactivity of these thiols. The evidence from Fig. 3 suggests that OPZ interacts with these highly reactive thiols on placenta GST. This was further supported by the inhibitory effects of OPZ on the binding of NEM to placenta GST. The inhibitory effects of OPZ and NEM on parasite GST were rather low compared to those on GST from human placenta. This suggests that thiols of parasite GST may be buried and less reactive as a result of protein conformation. Reaction of these GSTs with a denaturant, e.g. urea, before thiol assay would confirm this hypothesis and this pretreatment should result in more incorporation of both OPZ and NEM. The amount of [^{14}C]NEM incorporated into parasite

GST is lower than that bound to GST from human placenta, further suggesting low reactivity of SH groups on the former. This might also suggest that the SH groups of *S. mansoni* GSTs are neither part of the active site nor close to the active site. Although the amino acid sequence of parasite GST is known, the location of the enzyme active site is not known. The failure of RP 36 642 to inhibit GST or hexokinase activity or decrease binding of [^{14}C]NEM to protein SH groups suggests that the antischistosomal action of OPZ may be dependent on its ability to bind to SH groups of parasite macromolecules.

The covalent binding of OPZ to several proteins, particularly enzymes, may have deleterious effects on parasite physiology. Metabolic deficiency is likely to occur in the carbohydrate metabolic pathway since a number of enzymes, e.g. hexokinase, in this system are dependent on thiol reactivity [21]. The reaction of OPZ with thiols may now be used to explain various observations made earlier pertaining to some of the effects of the drug on schistosomes. Depletion of GSH [7, 9, 10], synergistic antischistosomal effects with cysteine and inactivity of certain OPZ analogues [7], increased bioavailability by co-administration with cysteine [33], blocking of OPZ-induced depression of schistosome surface and tegumental electrical potentials by incubation of parasites in the presence of GSH, cysteine and methionine and inhibition of cysteine uptake [9] are probably due to reactivity of OPZ with thiols. The inhibitory effects of OPZ [10] or its metabolites [11] on glutathione reductase activity and the dependency of such inactivation on NADPH suggests binding to the reduced enzyme form. The reactivity of OPZ *in vitro* with protein SH groups observed in the present study suggests that the unchanged form of OPZ may be responsible for its antischistosomal activity.

From the present results it is clear that the biochemical effects of OPZ are not restricted to parasite macromolecules. The selective accumulation of the drug in the parasites and the rapid first-pass metabolism by host liver [34] may be responsible for selective toxicity to the schistosomes. It was shown that parasites, particularly the females, accumulate OPZ levels to 10-fold those found in the plasma [34]. The concentration of OPZ used in the present study is comparable to that found in the parasite following treatment in infected mice. OPZ increases the levels of GSH and GST [35] in mammalian tissues. The elevated levels of GSH could be crucial in providing the essential SH groups to prevent or reverse the OPZ-induced inactivation of numerous mammalian enzymes during treatment of schistosomiasis with OPZ. It is not clear if the thioltransferase, which reductively cleaves protein disulfides [31], is involved in the regeneration of protein SH groups from OPZ–enzyme complexes. The levels of GSH in the parasite are, in general, very low [7], and OPZ-induced partial depletion of GSH would be more damaging to the schistosome compared to host tissues. In addition, the non-reversibility of the OPZ-induced parasite GST inactivation may further add to the toxicity of this drug to the parasite. These host–parasite differences may provide explanations for the selective toxicity of OPZ to the schistosomes. However, the possibility that OPZ reacts indiscriminately with SH groups raises serious questions on potential toxicity to human tissues.

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