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DIFFERENTIAL EFFECTS OF OLTIPRAZ AND ITS OXY-ANALOGUE ON THE VIABILITY OF *SCHISTOSOMA MANSONI* AND THE ACTIVITY OF GLUTATHIONE *S*-TRANSFERASE

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Abstract—Adult worms of *Schistosoma mansoni* recovered from mice treated with oltipraz (OPZ) showed a significant diminution in their ability to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan, a measure of parasite viability. Incubation of glutathione *S*-transferase (GST) from *S. mansoni* with OPZ resulted in a time- and concentration-dependent inhibition of enzyme activity. RP 36 642 (an inactive oxy-derivative of OPZ) had a minimal effect on the viability of the worms and no effect on GST activity. The structural integrity of OPZ, particularly the thione sulphur, appears to be necessary for expression of the antischistosomal effects of the drug. OPZ-induced inhibition of GST was non-competitive with either reduced glutathione (GSH) or 1-chloro-2,4-dinitrobenzene (CDNB), indicating that the drug is not a substrate for GST-catalysed conjugation reactions. In addition, the inhibition of GST could not be reversed by dialysis or repurification of the enzyme via a GSH-agarose affinity column. The effects of OPZ on GST activity could render the parasite vulnerable to damage by host-derived reactive oxygen species and aldehydic products of lipid peroxidation. The effects of OPZ on GST activity may play a role in the antischistosomal action of OPZ.

Oltipraz [4-methyl-5-(2-pyrazynl)-1,2-dithiole-3-thione] (OPZ)† is an antischistosomal compound that acts slowly to kill the major human infecting schistosomes [1–3]. The mechanisms of action of OPZ, although still under study, are thought to be related to its perturbation of reduced glutathione (GSH) metabolism. Leroy *et al.* [1] demonstrated that after treatment with OPZ of mice infected with *Schistosoma mansoni*, the adult worms showed reduced levels of GSH. This was followed by a decrease in the activities of glutathione *S*-transferase (GST) and glutathione reductase (GR) [4]. The mechanism by which OPZ decreases GST activity is not well understood. The effects of OPZ on GR activity, on the other hand, are known to be irreversible and dependent upon the metabolism of OPZ [5]. The effect of OPZ on other thiol-dependent enzyme systems has not been investigated.

GSH and its related enzymes have numerous functions including protection of tissues against damage by hydrogen peroxide and organic peroxides, via glutathione peroxidase (GPO) catalysed reactions. This results in the generation of oxidized glutathione (GSSG). GR prevents the accumulation of GSSG by catalysing its reduction back to GSH and thus maintains the redox state. GST consists of multifunctional isoenzymes that catalyse the conjugation of GSH with various xenobiotics and their metabolites as well as endogenously generated cytotoxic aldehydic products of lipid peroxidation. GST is important in binding several hydrophobic substances and thus facilitates their transport. GST has been associated with the development of drug resistance in tumours, insects and plants as well as parasites [6]. Parasite GST interacts with hematin, and this is thought to be important in preventing blockage of the intestines of blood-feeding parasites, such as schistosomes [7]. The selective inhibition of GST isoenzymes in the parasite could, therefore, lead to accumulation of toxic products, disruption of vital physiological processes, and the untimely death of the parasite. OPZ may exert some of its antischistosomal activity via such a phenomenon.

Although OPZ has been withdrawn from clinical trials [8], it is an important probe in investigating the effects of modulating GST activity on parasite survival, a recognized chemotherapeutic strategy. This article describes the nature and extent of OPZ-induced inhibition of GST from *S. mansoni*. The specificity of the inhibitory action of OPZ has been established by comparison with its oxy-analogue (RP 36 642). The data provide further insight into the basis for some of the antischistosomal activity of OPZ.

MATERIALS AND METHODS

Chemicals and reagents. All chemicals and reagents

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† Abbreviations: OPZ, oltipraz; GSH, reduced glutathione; GST, glutathione *S*-transferase; GR, glutathione reductase; GPO, glutathione peroxidase; GSSG, oxidised glutathione; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; DMSO, dimethyl sulfoxide; DMF, *N*,*N*-dimethylformamide; CDNB, 1-chloro-2,4-dinitrobenzene; and PBS, phosphate-buffered saline.

used were of analytical grade. OPZ (code: RP 35 972, lot: 6 CA 83 145 08) was provided by Rhone Poulenc Sante, Paris, France. The oxy-analogue of OPZ (code: RP 36 642, lot: PEA 453) was a gift from Dr. James L. Bennett, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI, U.S.A.

Exposure of parasites to drugs. A Puerto Rican strain of *S. mansoni* used in this study was maintained by cycling through *Biomphalaria glabrata* (Puerto Rican) snails and female CD-1 mice (Charles River, St. Constant, Quebec, Canada). Female CD-1 mice with 7- to 8-week-old infections of a Puerto Rican strain of *S. mansoni* were treated with 50 mg/kg of OPZ or RP 36 642. Each drug was made up as a suspension in a solution of 25% (v/v) glycerol and 1% (v/v) cremophor EL and administered by oral intubation. At various times after treatment, the mice were killed by cervical dislocation, and worms recovered by perfusion of the mesenteries were washed in Hanks' balanced salt solution and separated into males and females.

Assay for parasite viability. A simple three-step colorimetric quantitation assay based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was used to assess parasite viability [9]. The MTT assay is based on the selective uptake of the tetrazolium salt by viable/living cells followed by its reduction in the mitochondria to formazan, the coloured product. The amount of formazan produced gives a quantitative measure of viability. In this study, worms were placed individually into wells (96-well plates) containing 100 μL of phosphate-buffered saline (PBS) with 0.5 mg MTT/mL for 30 min at 37° (reduction). The solution was carefully removed and replaced with 200 μL dimethyl sulfoxide (DMSO). The worms were allowed to stand in DMSO at room temperature for 1 hr (solubilization) and absorbance was read at 550 nm relative to a DMSO blank. Heat-killed worms were used as controls.

Purification of GST from S. mansoni. Male *S. mansoni* worms were used as a source of material for the purification of GST. The worms were homogenized in 0.1 M potassium phosphate buffer containing 1 mM EDTA and centrifuged at 47,800 *g* for 1 hr followed by 90 min at 105,000 *g*. The supernatant (cytosolic fraction) was applied to a GSH-agarose affinity column [10]. The starting buffer, 22 mM potassium phosphate, pH 7, was used to wash through any unbound proteins. Bound proteins (GST) were eluted with 50 mM Tris·HCl, pH 9.6, containing 5 mM GSH. Fractions (2 mL) were collected and monitored for GST activity. Active fractions were pooled and dialysed against 10 mM potassium phosphate buffer, pH 7.0, containing 30% glycerol (storage buffer). The enzyme preparation was stored at −70° until needed.

GST inhibition assays. For the demonstration of time- and concentration-dependent inhibition of parasite GST by OPZ and RP 36 642, purified enzyme preparations were incubated for up to 1 hr with different concentrations of the drugs at 37°. OPZ and RP 36 642 were dissolved in *N,N*-dimethylformamide (DMF) and added as 10-μL solutions in a total volume of 500 μL. Incubations

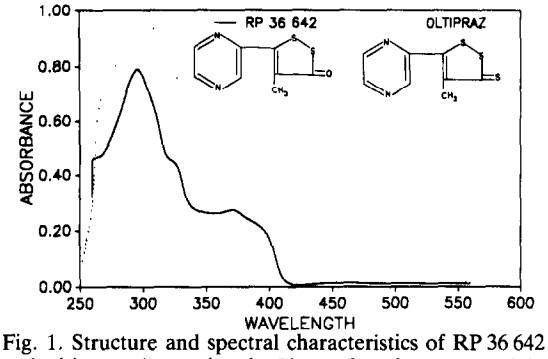

Fig. 1. Structure and spectral characteristics of RP 36 642 and oltipraz. Approximtely 80 μg of each compound in DMF was scanned at 200–900 nm in a DU-7 Beckman spectrophotometer. DMF alone was used as background. Results show the absorbance characteristics of the two drugs with their chemical structures above.

were carried out in 0.1 M potassium phosphate buffer. At various times during the incubation process, an aliquot containing 0.4 μg GST (50 μL) was removed and used for the determination of residual enzyme activity. Control incubations contained GST and DMF alone.

GST enzyme assay. GST activity was determined according to Habig *et al.* [11] using 0.1 M potassium phosphate, pH 6.5, 1 mM GSH and 1 mM 1-chloro-2,4-dinitrobenzene (CDNB). The reaction was initiated by a 50-μL enzyme solution in a total volume of 1 mL. The effect of OPZ on GST kinetic parameters (V_{max} and K_M) was determined using 0.5 and 1 mM OPZ (inhibitor). The nature of inhibition with respect to CDNB was determined with 1 mM GSH and various concentrations of CDNB (0.06 to 2 mM) in the GST assay mixture. Inhibition with respect to GSH was performed with 1 mM CDNB and GSH (0.06 to 2 mM). Apparent V_{max} and K_M values were obtained from Lineweaver–Burk plots.

Irreversibility of GST inhibition. The irreversibility of GST inhibition by OPZ was assessed by dialysis and repurification of the enzyme. For dialysis, GST (10 μg) was incubated with 0.5 mM OPZ for 1 hr at 37° followed by a 24-hr dialysis procedure using 10 mM potassium phosphate buffer, pH 6.5, containing 1 mM EDTA and 30% glycerol (v/v) at 4°. GST activity was assessed before and after the dialysis period. For repurification, GST was applied to a GSH-agarose column following a 1-hr incubation with OPZ. The column was washed thoroughly with the starting buffer before the elution procedure. Fractions (2 mL) were collected and monitored for GST activity. Control incubations did not contain OPZ.

Statistical analysis. The data were analysed for any statistical significance using the Dunnett's procedure or Student's *t*-test. P values less than 0.05 were declared significant.

RESULTS

Substitution of the thione sulphur with oxygen changed the absorbance characteristics of OPZ dramatically (Fig. 1). While RP 36 642 absorbed

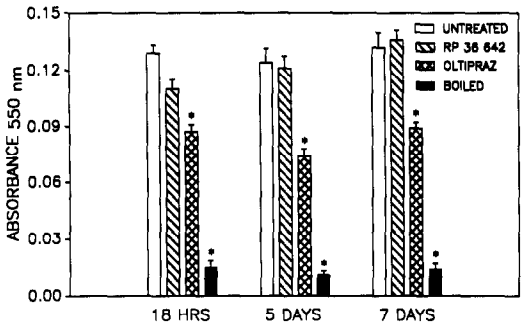

Fig. 2. Effects of OPZ and RP 36 642 on the ability of male *S. mansoni* to reduce MTT to formazan (measured at 550 nm). OPZ or RP 36 642 (50 mg/kg) was administered to *S. mansoni* infected female CD-1 mice by oral intubation. Control mice received the drug vehicle alone (glycerol/cremophor EL solution). The worms were harvested by perfusion of the mesenteries at 18 hr, 5 days, and 7 days after drug treatment. Male worms were separated from females and assessed individually for their ability to reduce MTT to formazan as described in Materials and Methods. At least ten worms were used in each determination, and the experiment was performed twice. Results are mean absorbance values per worm ± SEM. Key: (*) $P < 0.01$, control vs treated worms.

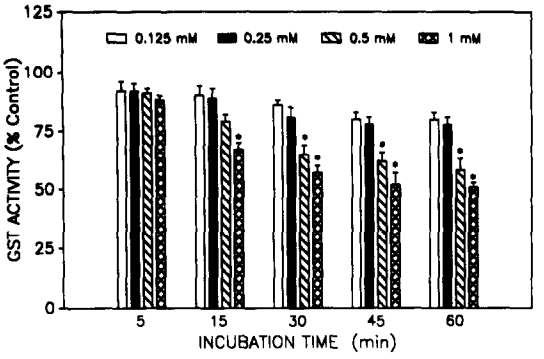

Fig. 3. Activity of purified GST from *S. mansoni* versus time of incubation at 37° with different concentrations of OPZ. OPZ was dissolved in DMF and added to the GST solution as 10 μL in a final volume of 500 μL. At various times, an aliquot (50 μL or 0.4 μg GST) was removed and assayed for GSI activity with GSH and CDNB. Results are mean percent control ± SEM of four separate determinations replicated three times. The GST activity in the control incubations was 29.55 ± 2.63 μmol/min/mg protein and did not decrease significantly after a 1-hr incubation period. Key: (*) $P < 0.05$ treated vs control for each time interval.

maximally near the UV range, OPZ had an additional peak in the visible range.

Effects of OPZ on the viability of S. mansoni. The effects of OPZ and the oxy-derivative RP 36 642, which lacks schistosomicidal action, on parasite viability are shown in Fig. 2. The presence of worms in 96-well plates did not affect readings at 550 nm. While OPZ caused a 42% drop in worm viability by 18 hr post-treatment, RP 35 642 induced a minimal decline (10%). Worms from animals treated with RP 32 642 showed full recovery by day 5 after treatment. Worms from the OPZ-treated mice, on the other hand, showed a 32% impairment in their ability to reduce MTT to formazan after 5 days. The dose of OPZ used (50 mg/kg) is subcurative in mice, but is sufficient to induce biochemical changes that are associated with change in parasite viability. When female parasites were studied, the MTT assay for parasite viability produced similar results as in the male worms (data not included).

Inhibition of S. mansoni *GST by OPZ.* Incubation of GST from *S. mansoni* with OPZ at 37° resulted in a concentration-dependent inhibition of enzyme activity (Fig. 3). A significant decline in GST activity was obtained with higher concentrations of OPZ after about 30 min. Incubation of GST with OPZ for an extended period of time did not result in further inhibition of activity. RP 36 642 had no effect on the activity of *S. mansoni* GST even when used at a 2 mM final concentration in the incubation assays (Fig. 4). Thus, RP 36 642 had little or no effect on the viability of the worms (Fig. 2), or on the activity of GST (Fig. 4). The kinetics of *S. mansoni* GST activity in the presence of OPZ (inhibitor) displayed non-competitive inhibition with respect to the function of the concentration of both CDNB (Fig. 5a) and GSH (Fig. 5b). There was a

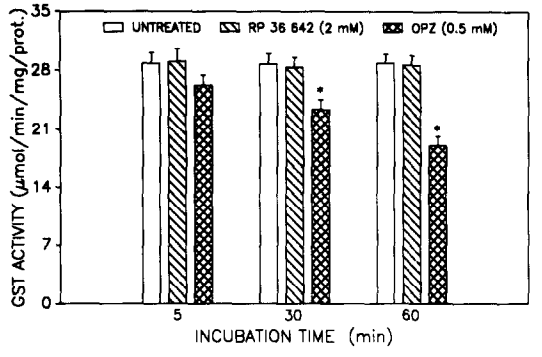

Fig. 4. Effects of OPZ and RP 36 642 on the activity of GST from *S. mansoni*. GST purified from male parasites was incubated with OPZ (0.5 mM) or RP 36 642 (2 mM) for up to 60 min. At 5, 30 and 60 min during the incubation period, an aliquot was removed and used for analysis of GST activity. Values are means ± SEM, N = 4. Key: (*) differs significantly from the activity in the absence of the drug ($P < 0.05$).

significant reduction in V_{max} after incubation of GST with OPZ, while the K_M values were essentially unchanged. Similar kinetic effects were observed when using GST from worms recovered from mice treated with OPZ (unpublished observation).

Effect of dialysis and repurification on OPZ-inactivated GST. The incubation of GST with OPZ (0.5 mM) resulted in a 30% inhibition of activity; extensive dialysis (equivalent to 1:2000 dilution of OPZ in the assay) did not reverse the inactivation (Fig. 6). The GST activity remained inhibited by about 31% after dialysis. During the dialysis period, GST lost some activity not attributable to the effects of OPZ. To confirm the irreversible effects of OPZ,

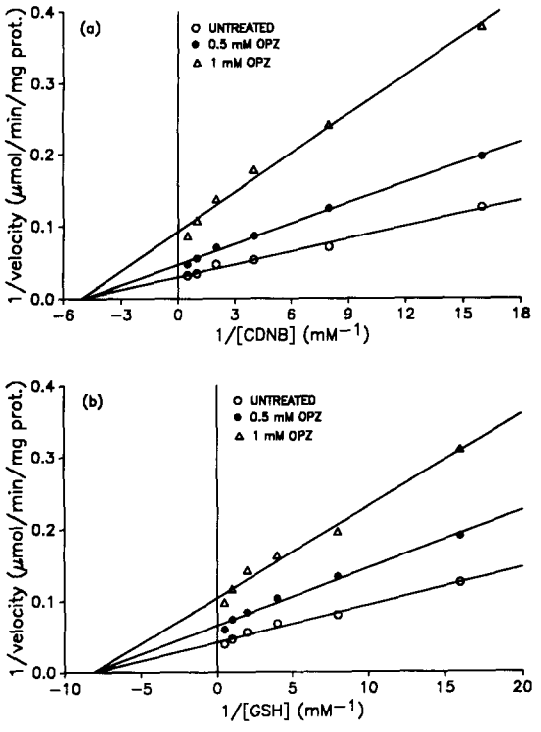

Fig. 5. Lineweaver–Burk plots showing non-competitive inhibition of GST by OPZ towards both CDNB (a) and GSH (b). GST was incubated for 60 min with OPZ prior to assessment of activity with different concentrations of either CDNB or GSH as described in Materials and Methods. Each graph is a representative of at least three determinations.

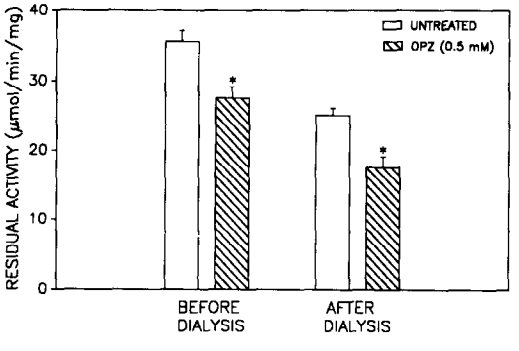

Fig. 6. Effects of dialysis on residual activity of GST from *S. mansoni* that had been inactivated by OPZ. GST was incubated with OPZ (0.5 mM) for 60 min at 37°. Approximately 1 mL of the solution with or without OPZ was dialysed for 24 hr at 4° in 1 L of 0.1 M potassium phosphate buffer, pH 6.5, with 1 mM EDTA and 30% glycerol. Results are expressed as remaining activity ± SE of three determinations. Key: (*) $P < 0.05$, control vs OPZ-containing sample.

inactivated GST was loaded onto a GSH-agarose affinity column. After extensive washing with the starting buffer and elution, the GST activity still remained inhibited (data not shown). Preincubation of GST with OPZ did not prevent the enzyme from binding onto the GSH-column as evidenced by the absence of GST activity in breakthrough fractions.

DISCUSSION

The metabolism of MTT to formazan has been widely applied as a quantitative measure of cell viability [12] particularly in assessing proliferation and cytotoxicity in anticancer chemotherapy. The application of this assay to helminths was pioneered by Comley *et al.* [9], and several nematode species were assessed. In comparison with other more specific biochemical parameters for parasite viability, the MTT assay was in good agreement [13]. In schistosome research, viability after exposure of worms to cytoxic immune cells or drugs has most often been assessed by microscopically evaluating motility and the morphological integrity of the parasites. The inherent subjectivity of these methods led us to utilize the MTT-formazan assay to compare the effects of OPZ and RP 36 642 on schistosomes. It yielded quantatitive data on the functional status of the worms, was simple, and could be performed rapidly.

Although OPZ is a slow-acting compound, some of its biochemical effects are manifested early after treatment and these may account for the final death of the parasites. The MTT assay has been used to detect some of the early effects of OPZ. One such early biochemical effect of OPZ on the parasites is the lowering of GSH levels [2] and this has been detected as early as 1 hr after treatment [14]. OPZ also results in a decrease in the activities of GST and GR [4]. RP 36 642, on the other hand, does not affect the levels of GSH [2] and here we have demonstrated that it has no effect on the viability of the parasites either. This suggests that thione sulphur is a crucial determinant in the induction of early drug effects on the parasite. In general, compounds with antischistosomal action cause a shift in the location of parasites from the mesenteric veins (normal habitat) to the liver. This hepatic shift [15] is normally preceded by early and important primary drug effects. Primary drug effects that result in hepatic shift after OPZ treatment may include lowering of the levels of GSH and the activities of GST and GR. Therefore, the effects of OPZ on GST were studied *in vitro*.

It is difficult to explain the exact mechanism of action of antiparasitic drugs because they often induce a plethora of biochemical effects on the parasite. The mechanism of action of OPZ is not clear either, but its profound effect on glutathione metabolism seems to be central to its antiparasitic effects. This study provides evidence that the effect of OPZ on GST activity resides in the parent molecule. The effects of OPZ on GR, on the other hand, are dependent on the drug being metabolized first [5]. Relatively high levels of OPZ were used in this study to achieve significant and relatively rapid inhibition of GST activity *in vitro*. In the host, the

location of *S. mansoni* (mesenteric veins) allows the worms to encounter and take up large amounts of OPZ following oral administration. Schistosomes concentrate OPZ by a 2- to 10-fold factor greater than the levels present in plasma in the first 12 hr after administration [16], and the mechanism involved in such accumulation has not been investigated. The unchanged form of the drug has a long retention time in the parasite, and there is no evidence that schistosomes are able to metabolize OPZ. In animals [5] and humans [17], OPZ is rapidly metabolized and cleared from circulation with plasma elimination half-lives of 2.5, 4.5 and 3.5 to 7 hr in rats, mice and monkeys, respectively [16]. The concentration of OPZ found in the parasite *in vivo* following drug treatment and that employed in this *in vitro* study are comparable. Thus, the direct inhibition of GST by OPZ *in vivo* is conceivable. Although OPZ also inhibited host (mammalian) GST *in vitro* in an irreversible manner (data not shown), its effects *in vivo* are likely to be curtailed by the rapid induction of drug-metabolizing enzymes, including GST and GR, as well as elevating GSH concentrations [18]. Administration of phenobarbital, an inducer of drug-metabolizing enzymes, to mice prior to treatment with OPZ reduces the antischistosomal action of OPZ [2]. The accumulation of inhibitory levels of OPZ in mammalian tissues is, therefore, unlikely.

The kinetics of GST inhibition by OPZ both *in vivo* and *in vitro* were similar, characterized by a significant decrease in V_{max} but not K_M values. The specificity of GST–OPZ interaction has been established by the use of RP 36 642, in which the thione sulphur is replaced by oxygen (Fig. 1). Taken together, these results suggest that the effects of OPZ on GST activity *in vivo* may not be dependent on its metabolism. The effect of OPZ on GST activity is probably due to a specific drug–enzyme interaction that produces non-competitive inhibition. Such an interaction appears to be non-reversible and, thus, may remain in place *in vivo* until the death of the parasite. Previous studies demonstrated that worms treated *in vivo* with OPZ may lose up to 70% of their GST activity by day 10 after such treatment.*

The impairment of GST and GR activities together with the lowering of the levels of GSH may lead indirectly to parasite death. The ultimate death of the parasite is probably due to the toxicity of reactive oxygen species from immune cells and the lethal action of cytotoxic aldehydic products of lipid peroxidation. The levels of thiobarbituric acid-reactive substances (indicators of lipid peroxidation) were found to increase after OPZ treatment [4]. The 4-hydroxyalk-2-enal aldehydes, known products of lipid peroxidation, were found to be toxic to *S. mansoni in vitro* and they are synergistic with reactive oxygen species in parasite killing [4]. The 4-hydroxyalk-2-enals inhibit GST activity [4] and have been demonstrated to be excellent substrates for GST from parasites [19]. The impairment of GST activity in the face of increased generation of toxic metabolites may render the parasite defenseless, resulting in tissue damage and death.

The physical and antischistosomal characteristics of OPZ seem to depend on the thione sulphur. It is probable that this sulphur reacts with sulphydryl groups in proteins, particularly enzymes. Enzymes with exposed cysteine residues are likely to be affected. The effects of OPZ on thiols could be disruptive to enzymes that depend on the oxidation and reduction of their thiol groups for metabolic regulation. However, it is not yet clear whether OPZ has general effects on thiol-dependent enzymes. The GSH-cycle is an important parasite defense system against xenobiotics and locally generated reactive metabolities. The ability of OPZ to modulate GSH metabolism may be the basis for the drug's antiparasitic action, and provides a model system for the study of the effects of GSH perturbation on parasite viability/survival. The specific impairment of GST, a multifunctional protein that is vital to the parasite, is probably a major contributor to the antischistosomal action of OPZ.

* Nare B, Smith JM and Prichard RK, Oltipraz-induced decrease in the activity of cytosolic glutathione *S*-transferase in *Schistosoma mansoni*, in press.

REFERENCES

1. Leroy JP, Barreau M, Cotrel C, Jeanmart C, Messer M and Benazert 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. 1, 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. Mkoji GM, Smith JM and Prichard RK, Glutathione redox state, lipid peroxide levels, and the activities of glutathione enzymes in oltipraz-treated adult *Schistosoma mansoni*. *Biochem Pharmacol* **38**: 4307–4313, 1989.
5. Morreau N, Martens T, Fleury MB and Leroy JP, Metabolism of oltipraz and glutathione reductase inhibition. *Biochem Pharmacol* **40**: 1299–1305, 1990.
6. Clark G, The comparative enzymology of the glutathione *S*-transferases from non-vertebrate organisms. *Comp Biochem Physiol* **92B**: 419–446, 1989.
7. Howell MJ, Board PG and Boray JC, Glutathione *S*-transferase in *Fasciola hepatica*. *J Parasitol* **74**: 715–718, 1988.
8. Sturrock RF, Oltipraz withdrawn from clinical trials. Correspondence. *Trans R Soc Trop Med Hyg* **81**: 528, 1987.
9. Comley JCW, Rees MJ, Turner CH and Jenkins DC, Colorimetric quantitation of filarial viability. *Int J Parasitol* **19**: 77–83, 1989.
10. Simons PC and Vander Jagt DL, Purification of glutathione *S*-transferase from human liver by glutathione affinity chromatography. *Anal Biochem* **82**: 334–341, 1977.
11. 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.

12. Mosmann T, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**: 55–63, 1983.
13. Comley JW, Rees MJ and O'Dowd AB, The application of biochemical criteria to the assessment of macrofilarial viability. *Trop Med Parasitol* **39**: 456–459, 1988.
14. Morrison DD, Thompson DP, Semeyn DR and Bennett JL, Acute effects of oltipraz on adult *Schistosoma mansoni* and its antagonism *in vitro*. *Biochem Pharmacol* **36**: 1169–1171, 1987.
15. Stranden OD, Experimental schistosomiasis III: Chemotherapy and mode of drug action. *Ann Trop Med Parasitol* **47**: 26–43, 1953.
16. Heusse D, Marlard M, Bredenbac J, Decouvelaere B, Leroy JP, Bieder A and Humeau H, Disposition of ^{14}C-oltipraz in animals: Pharmacokinetics in mice, rats and monkeys. Comparison of the biotransformation in the infected mouse and in the schistosomes. *Arzneimittelforschung (Drug Res)* **35(II)**: 1431–1436, 1985.
17. Homeida MMA, Hassan MA, Suad MS and Bennett JL, Oltipraz administration with food increases its antischistosomal activity. *Trans R Soc Trop Med Hyg* **80**: 908–910, 1986.
18. Davies MH, Blacker AM and Schnell RC, Dithiolethione-induced alterations in hepatic glutathione and related enzymes in male mice. *Biochem Pharmacol* **36**: 568–570, 1987.
19. Brophy PM and Barrett J, Glutathione transferases in helminths. *Parasitology* **100**: 345–349, 1990.