

Inhibition of Filarial Glutathione-S-transferase by various classes of compounds and their evaluation as novel antifilarial agents

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Summary

Glutathione S-transferase(s); GST(s) (E.C. 2.5.1.18) are a large family of multifunctional dimeric enzymes that conjugate reduced glutathione to electrophilic centres in hydrophobic organic compounds. GST(s) represent the major class of detoxifying enzymes from parasitic helminths. The GST enzymatic activity has been described in the adult and larval stages of helminths. Several forms and isoforms of the enzyme have been purified and GST genes have also been isolated and expressed as recombinant proteins. The helminth GST(s) participate in detoxification of lipid hydroperoxides and cytotoxic carbonyl compounds produced by oxygen-reactive intermediates (ORIs). The ORIs can come from the endogenous parasite metabolism or from the host immune system. The helminth GST(s) are able to conjugate glutathione to xenobiotic compounds or to bind to the anthelmintic drugs. GST is usually found to be localized near to host-parasite interface. This enzyme has been identified as a potentially vulnerable target in immunotherapy and chemotherapy of parasitic diseases. The most effective drug candidates are those based on inhibitors of GST. In the present study, purified GST from cytosolic fraction of bovine filarial worms *Setaria cervi* was inhibited in a concentration dependent fashion by various compounds such as hemin, ethacrynic acid, S-hexylglutathione, quercetin, cibacron blue, lithocholate sulfate and ellagic acid. Cytosolic GST was inhibited to varying degrees by each inhibitor. In this context, the possible physiological significance of the observed results has been discussed.

Key words: Glutathione-S-transferase; enzyme inhibition; glutathione analog; *Setaria cervi*; helminth parasites; anti-parasitic agents

Introduction

Glutathione-S-transferase(s); GST(s) (E.C. 2.5.1.18) are a multigene family of enzymes catalyzing the conjugation of glutathione with a large variety of xenobiotics as well as endogenous substrates. The main functions of these enzymes is to bring the substrate into close proximity with GSH by binding both GSH and electrophilic substrate (R-X) to the active site of the protein and activate the sulfhydryl group on GSH, thereby allowing the nucleophilic attack of GSH on the electrophilic substrate (R-X) (Armstrong, 1997). In GST(s) catalyzed reactions, the sulphur atom of GSH acts as nucleophilic substrate. The resulting GSH conjugate is then either excreted directly or further metabolized to yield a mercapturic acid (Meister & Anderson, 1983). GST(s) catalyze various other reactions like nucleophilic aromatic substitution reactions, reversible Michael additions to α , β -unsaturated aldehydes and ketones, isomerization, epoxide ring openings and for few GST(s), peroxidase reactions (Eaton & Banunler, 1999; Salinas & Wong, 1999). In addition to their isomerization and GSH conjugation activities, in mammals these enzymes contribute to defence against oxidative stress, by virtue of both their selenium-independent GSH peroxidase activities (Zhao *et al.*, 1999). One prominent feature of GST is the presence of a number of isoenzymes in a given species (Mannervik & Danielson, 1988). Kinetic properties such as inhibitor sensitivities and substrate specificities have been used to distinguish different GST isoenzymes. The GST enzyme family has been divided into a number of classes based on various criteria, such as amino acid/nucleotide sequence, immunological, kinetic and tertiary/quaternary structural properties (Sheehan *et al.*, 2001). Eukaryotic GST(s) are currently arranged into 12 different classes: alpha, delta, epsilon, lambda, mu, omega, phi, pi, sigma, tau, theta and zeta as well as a mitochondrial kappa and a microsomal class (Gulick & Fahl, 1995; Chelvanayagam *et*

al., 2001; Sheehan *et al.*, 2001; Marco *et al.*, 2004; Hayes *et al.*, 2005). Most well studied species have been shown to have genes for several of these GST classes, although some classes are still considered to be taxon-specific (e.g. phi, tau and lambda have been found only in plants whereas delta and epsilon only in insects).

These enzymes exist as dimeric proteins comprised of two subunits (Cnubben *et al.*, 2001). Each of the subunits possesses a glutathione-binding site (G site) as well as an adjacent relatively hydrophobic site for the binding of the electrophilic substrate. In addition, in various isoenzymes (alpha and pi class) a non-substrate binding site has been recognized, which may function as a transport site or a regulatory site (van Bladeren, 2000).

Inhibition of GST(s) is important from several points of view. These involve applications in studies of the catalytic mechanism, e.g. studying the topological and binding characteristics of the active site. Also, from a therapeutic point of view, inhibition of GST(s) steadily becomes more interesting, since these enzymes appear to be involved in drug resistance and in the biosynthesis of a number of important arachidonic acid metabolites such as prostaglandins and leukotrienes (van Bladeren & Ommen, 1991). Modulation of the GST activity could be used to regulate the concentrations of these compounds. Towards this end, many research groups have been pursuing the development of GST inhibitors for more than a decade (Flatgaard *et al.*, 1993; Lyttle *et al.*, 1994; Morgan *et al.*, 1996; Lyon *et al.*, 2003). Also, because of its role in protecting cells against immune mediated lipid peroxidation, inhibition of GST offers the possibility of combining immuno- and chemotherapy. In diagnostic medicine, as well as in antiparasitic drug development, GST inhibitors are important lead molecules (Mathew *et al.*, 2006).

Glutathione metabolism represents a potential target for antiparasite drug design. GST, an important enzyme of GSH cycle, is considered to be an essential detoxification enzyme in parasitic species. The interest in helminth GST(s) stems from the fact that they represent the major detoxification enzymes in these organisms and consequently represent important pharmacological and immunological targets (Morrison *et al.*, 1996). As helminths contain very low levels of other detoxification enzymes such as catalase, superoxide dismutase (SOD) and cytochrome P450, GST(s) may provide the worm's primary defense against electrophilic and oxidative damage (Brophy & Barrett, 1990). Primary amino acid sequence alignment of helminth GST(s) with mammalian GST(s) indicates that non-mammalian GST(s) superfamilies are represented in these parasites. Helminth GST(s), though present as isoenzymes, fail to show a clear biochemical homology to any of the three main mammalian GST families (O'Leary & Tracy, 1988; Brophy & Barrett, 1990). Thus, inhibition of helminth GST(s) by compounds may be advantageous from the host's point of view as their inhibition deprives the parasites of their major defense against oxidative stress and makes them unable to survive. In the present work we have investigated the relative susceptibi-

lity of GST from bovine filarial worms *Setaria cervi* to inhibition by various classes of mammalian GST inhibitors.

Materials and Methods

Materials

Reduced glutathione (GSH), trizma, ethylene diamine tetra acetic acid (EDTA), potassium chloride (KCl), sodium chloride (NaCl), hemin, ethacrynic acid (EA), S-hexylglutathione, quercetin, cibacron blue (CB), lithocholate sulfate (LS), ellagic acid and bovine serum albumin (BSA) were purchased from Sigma Chemical Co., USA. 1-chloro-2, 4-dinitrobenzene (CDNB) was from Spectrochem Pvt. Ltd. Mumbai, India. Folin & Ciocalteu's phenol reagent was purchased from Sisco Research Laboratories, Mumbai, India. Hanks balanced salt solution (HBSS) was from Himedia laboratories Pvt. Ltd. Mumbai, India. All other chemicals used were of analytical grade.

Filarial worms

In view of the practical difficulties in procuring human strains of *Wuchereria bancrofti* and *Brugia malayi*, the bovine filarial parasite *S. cervi*, resembling the human species in having microfilarial periodicity and chemotherapeutic response to known antifilarial agents, was chosen as a model for conducting enzymatic studies.

Collection of filarial worms *S. cervi*

Adult bovine filarial worms *S. cervi* females of average body weight 35 ± 5 mg and length 6.0 ± 1.0 cm were collected from the peritoneal cavity of freshly slaughtered naturally infected water buffaloes *Bubalus bubalis* (Linn.) at a local abattoir. They were thoroughly washed with saline and kept in Hanks balanced salt solution with sodium bicarbonate and glucose (5.55 mM) for 60 min at 37 °C in a Dubnoff metabolic shaker for complete revival before being used in enzymatic studies.

Preparation of GST from *S. cervi*

A 10 % homogenate of filarial worms *S. cervi* was prepared in 50 mM Tris-HCl buffer, pH 7.4 containing 250 mM sucrose and 0.2 mM EDTA using a Potter Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 1,000 g for 15 min, 10,000 g for 30 min and subsequently at 100,000 g for 60 min to obtain mitochondrial, post mitochondrial, cytosolic and microsomal fractions respectively.

Each fraction was dialyzed against 100 volumes of 20 mM potassium phosphate, pH 7.4 containing 0.2 mM EDTA and dialyzed preparation was used as enzyme source.

GST activity determination

GST activity in various dialyzed fractions obtained as a result of subcellular fractionation was determined spectrophotometrically at 340 nm according to the method of Habig *et al.* (1974). All reactions were corrected for non-enzymatic conjugation, with reaction mixtures without

enzyme serving as controls.

Under standard assay conditions, the reaction mixture contained 100 mM phosphate, pH 6.5, 1.0 mM CDNB in 20 μ l ethanol, 1.0 mM GSH and 25 μ l enzyme preparation containing at least 62.50 μ g \pm 0.025 protein unless stated otherwise. A unit of enzyme activity was expressed as the amount that catalyzes the formation of 1 μ mol S-2, 4-dinitrophenyl-GSH adduct per minute, using a molar extinction coefficient of 9.6 mM⁻¹ cm⁻¹ for CDNB. Protein was estimated by the method of Lowry *et al.* (1951) using BSA as standard.

Purification of GST from *S. cervi*

In the standardized protocol, purification was carried out by the preparation of the homogenate, salt precipitation of the cytosolic fraction, ultrafiltration, ion exchange chromatography and affinity chromatography on GSH-Sepharose. The GSH affinity column was prepared according to the method of Simons and Vander Jagt (1977). Native PAGE was performed on a 7.5 % gel according to the method of Laemmli, (1970). Gels were stained for GST activity after native PAGE using the method of Ricci *et al.* (1984). GST purification was optimized by inclusion of relatively fewer steps to get a better yield of the enzyme. The homogeneity of the purified protein was confirmed by SDS-PAGE analysis and MALDI TOF studies. Two-dimensional electrophoresis of the purified GST yielded a single spot thereby suggesting that *S. cervi* has only a single GST (results not shown).

Inhibition studies

In order to determine dose-dependent effect of various inhibitors and their respective inhibitor constants (K_i), the purified enzyme was incubated with varying concentrations of inhibitor for 10 min at room temperature in the presence of 100 mM potassium phosphate buffer and 1.0 mM GSH. The reaction was initiated by the addition of 1.0 mM CDNB and the absorbance at 340 nm was monitored for 5 min at 30 sec intervals. The percentage inhibition of the enzyme activity by various inhibitors was calculated by comparing with a control tube. Decrease in enzyme activity in presence of varying concentrations of inhibitors as compared to control was expressed as μ mol S-2, 4-dinitrophenyl-GSH adduct formed/min \pm S.D. based on experiments done in quadruplicates.

For determining the type of inhibition of *S. cervi* GST with respect to GSH and CDNB by a particular inhibitor, the purified cytosolic enzyme was incubated with varying concentrations of inhibitors at different concentrations of one substrate for 10 min at room temperature in the presence of 100 mM potassium phosphate buffer while keeping the concentration of the other substrate fixed. The reaction was initiated by addition of CDNB and the absorbance at 340 nm was monitored for 5 min at 30 sec intervals.

In order to determine IC₅₀ values (inhibitor concentration at which enzyme inhibition was 50 %) of various inhibitors and their respective inhibitor constants (K_i), enzyme was incubated with varying concentrations of inhibitor for 10 min at room temperature in the presence of 100 mM potassium phosphate buffer and 1.0 mM GSH. The reaction was initiated by the addition of 1.0 mM CDNB and the absorbance at 340 nm was monitored for 5 min at 30 sec intervals. The percentage inhibition of the enzyme activity by various inhibitors was calculated by comparing with control tube.

$$\text{Percent inhibition} = \frac{\text{OD/min of control tube} - \text{OD/min of experimental tube} \times 100}{\text{OD/min of control tube}}$$

Results

Table 1 depicts the distribution of GST activity in various subcellular fractions of *S. cervi* homogenate. It is evident from the results that GST activity in these worms was also mainly associated with the cytosolic fraction, which was used as source for enzyme purification. Tetrapyrroles (bilirubin, biliverdin, chlorophyllin and hemin) are known inhibitors of GST. Chlorophyllin and hemin are the most effective in inhibiting the activity of GST whereas bilirubin and biliverdin are the least effective. Thus, the degree of inhibition is related to whether the tetrapyrroles are closed or open chain compounds. Chlorophyllin and hemin are closed chain tetrapyrroles whereas bilirubin and biliverdin are open chain tetrapyrroles. Ferriprotoporphyrin IX chloride (hemin), a known inhibitor of mammalian GST inhibited *S. cervi* GST in a concentration dependent fashion when studied for its effect in the 10 to 50 μ M range. It maximally inhibited GST to around 92.4 % at 50 μ M

Table 1. Subcellular distribution of GST activity in filarial worms *S. cervi*

Fraction	Total activity* (μ mol/min)	Total protein (mg)	Specific Activity** (μ mol/min/mg protein)
Crude homogenate	1.40 \pm 0.090	20.1 \pm 0.152	0.070 \pm 0.0185
Mitochondrial fraction	0	1.25 \pm 0.080	Not detectable
Post mitochondrial fraction	1.20 \pm 0.042	18.2 \pm 0.056	0.066 \pm 0.017
Cytosolic fraction	2.024 \pm 0.018	13.9 \pm 0.033	0.122 \pm 0.024
Microsomal fraction	0.026 \pm 0.02	2.54 \pm 0.025	0.010 \pm 0.0052

*Activity was expressed as μ mol S-2, 4-dinitrophenyl-GSH adduct formed/min \pm S.D. based on experiments done in quadruplicates.

** Specific Activity was expressed as μ mol S-2, 4-dinitrophenyl-GSH adduct formed/min/mg protein \pm S.D. based on experiments done in quadruplicates.

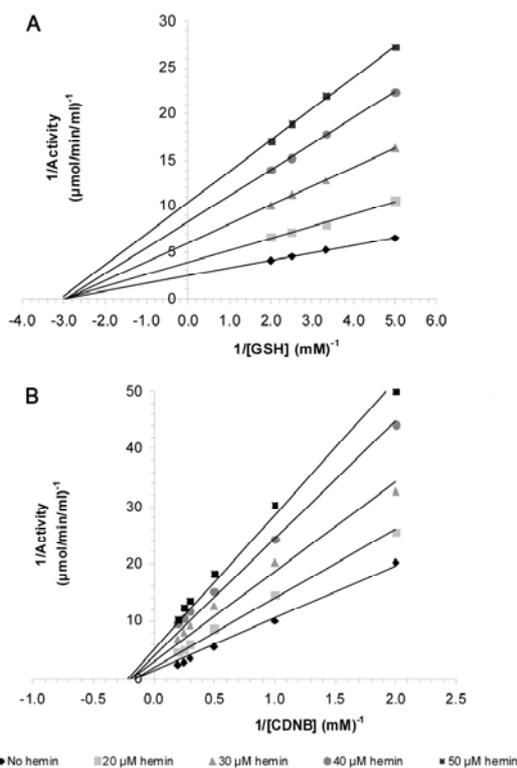


Figure 1. Type of inhibition on the activity of purified GST from *S. cervi* with respect to GSH (A) and/or CDNB (B) by hemin.

Decrease in enzyme activity in the presence of varying concentrations of inhibitor as compared to control was expressed as $\mu\text{mol S-2, 4-dinitrophenyl-GSH adduct formed/min} \pm \text{S.D.}$ based on experiments done in quadruplicates.

and was therefore found to be a potent inhibitor of *S. cervi* GST. Figures 1a and b depict the GST catalyzed conjugation of GSH to CDNB in the presence and absence of hemin. Table 2 depicts the IC_{50} value and apparent K_i of the enzyme with respect to hemin. The phenoxy acetic acid diuretic, ethacrynic acid, also

inhibited *S. cervi* GST activity in a concentration dependent fashion when studied for its effect in the concentration range 2.0 to 40 μM . It maximally inhibited GST to around 72.1 % at 40 μM . Figures 2a and b, respectively depict the pattern of inhibition of the enzyme with respect to substrates GSH and CDNB by ethacrynic acid. Table 2 gives the IC_{50} value and K_i value of the enzyme with respect to ethacrynic acid.

The GSH analog *S*-hexylglutathione inhibited purified GST from *S. cervi* in a concentration dependent manner when studied for its effect in the concentration range 0.025 to 0.2 mM. It maximally inhibited GST to around 90.2 % with respect to substrate GSH (Figure 3).

The flavonoid inhibitor quercetin also inhibited *S. cervi* GST activity in a concentration dependent fashion when studied for its effect in the concentration range 10 to 50 μM . GST was maximally inhibited to around 82 % at 50 μM concentration of quercetin.

The dye cibacron blue (CB) inhibited the purified enzyme in a concentration dependent fashion in the concentration range 0.0125 to 0.25 μM . Maximum inhibition on GST activity was around 92.5 %.

Bile acid lithocholate sulfate (LS) inhibited cytosolic GST from *S. cervi* within the concentration range 0.1 – 1.0 mM with a maximum inhibition of 90.3 %.

The antioxidant plant phenol ellagic acid exhibited a maximum inhibition of 78.1 % on purified cytosolic GST activity when studied in the concentration range 0.1 – 1.5 μM . Of all inhibitors tested, hemin was found to be a potent inhibitor of *S. cervi* GST. Further studies into the structure and binding properties of hemin to GST may be of critical importance for the development of therapeutic agents.

Discussion

A large variety of inhibitors of GST(s) from mammalian sources are known. Some of these are strong and selective enough to be used to characterize individual isoenzymes

Table 2. K_i and IC_{50} values of standard GST inhibitors with respect to cytosolic GST from *S. cervi*

Inhibitor	Class	K_i value	IC_{50} value
Hemin	porphyrin	$4.0 \pm 0.08 \mu\text{M}$	$25.0 \pm 0.03 \mu\text{M}$
Ethacrynic acid	Substituted phenoxy acetic acid	$22 \pm 0.125 \mu\text{M}$	$15 \pm 0.110 \mu\text{M}$
<i>S</i> -hexylglutathione	GSH analog	$0.04 \pm 0.01 \text{mM}$	$0.1 \pm 0.008 \text{mM}$
Quercetin	Flavone derivative	$5.0 \pm 0.05 \mu\text{M}$	$25 \pm 0.15 \mu\text{M}$
Cibacron blue-3GA	Dye	$0.02 \pm 0.006 \mu\text{M}$	$0.025 \pm 0.01 \mu\text{M}$
Lithocholate sulfate	Bile acid	$0.1 \pm 0.005 \text{mM}$	$0.3 \pm 0.002 \text{mM}$
Ellagic acid	Substituted lactone derivative	$0.27 \pm 0.004 \mu\text{M}$	$0.8 \pm 0.002 \mu\text{M}$

K_i and IC_{50} value were calculated by measuring enzyme activity in the presence of varying concentrations of inhibitors as $\mu\text{mol S-2, 4-dinitrophenyl-GSH adduct formed/min} \pm \text{S.D.}$ based on experiments done in quadruplicates

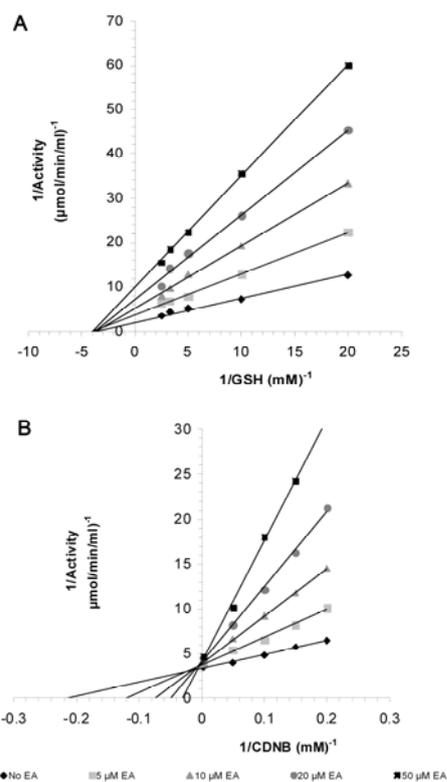


Figure 2. Type of inhibition on the activity of purified GST from *S. cervi* with respect to GSH (A) and/or CDNB (B) by EA. Decrease in enzyme activity in the presence of varying concentrations of inhibitor as compared to control was expressed as $\mu\text{mol S-2, 4-dinitrophenyl-GSH adduct formed/min} \pm \text{S.D.}$ based on experiments done in quadruplicates.

(Mannervik, 1985). GST inhibitors that are relatively non-toxic, isoenzyme specific and active *in vivo* have not yet been developed. Despite a considerable effort, so far the only *in vivo* active inhibitors of GST(s) are ethacrynic acid and a number of glutathione derived structures (Morgan *et al.*, 1996; Mulder & Ouwkerk-Mahadevan, 1997). A number of inhibitors have been reported that are suitable for *in vitro* studies. These include ethacrynic acid, the antibiotic calvatic acid, curcumin, haloenol lactone, disulfiram and organotin compounds, to name just a few (Morgan *et al.*, 1996; van Iersel *et al.*, 1996, 1997).

The currently existing GST inhibitors tend to fall into three classes, determined by their binding site on the protein and mechanism of inhibition. The first of these are analogs of electrophilic substrates that bind in the hydrophobic region of the active site (H-site) and competitively inhibit the binding of hydrophobic electrophiles. Second are glutathione conjugates which occupy both the glutathione binding site (G-site) and at least part of the H-site and are typically competitive with respect to both GSH and hydrophobic substrate. Third, a collection of compounds referred to as nonsubstrate ligands are non-competitive inhibitors of GST(s). These inhibitors are typically hydrophobic anions and include endogenous compounds such as porphyrins and bile acids and such exogenous compounds as sulfated organic dyes. The binding site of this group of inhibitors has historically been called the ligandin site and its loca-

tion remains uncertain; however, it may lie at one edge of the H-site or within the wide cleft between the two subunits of the GST dimer. These nonsubstrate ligands are unique among the GST inhibitors in that they elicit non-competitive inhibition with respect to both GSH and electrophilic substrate.

The cytosolic enzyme from *S. cervi* exhibited inhibition with various inhibitors used to distinguish the mammalian GST families but showed no clear biochemical relationship to any one mammalian GST class. Although class-distinguishing substrates and inhibitors are useful, it has been demonstrated that isolated GST(s) have overlapping substrate specificities and no single compound can give an unambiguous distinction (Mannervik *et al.*, 1985). The inhibitor profile of *S. cervi* GST showed a limited relationship to the profile of mammalian GST(s) (Stockman *et al.*, 1987). The inhibitor profile of *S. cervi* GST is similar to that described for the *Schistosoma mansoni* (*Sm28*) GST (Walker *et al.*, 1993), *O. volvulus* GST (*OvGST2*) (Liebau *et al.*, 1996) and GST from *Ascaris suum* (Liebau *et al.*, 1997). From the perspective of classification, it is interesting that, whereas helminth organisms express GST(s) that are clearly related to mu, pi and sigma classes of other organisms, these enzymes contain sufficient regions of structural difference compared with host enzymes to hold out the prospect of development of parasite-specific vaccines.

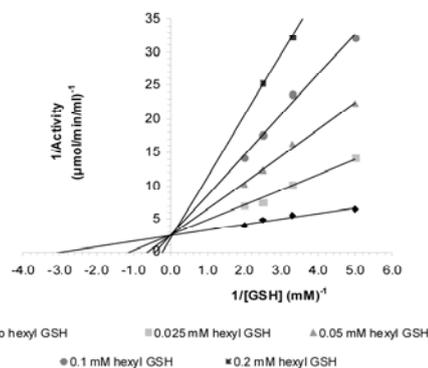


Figure 3. Type of inhibition on the activity of purified GST from *S. cervi* with respect to CDNB by hexylglutathione. Decrease in enzyme activity in the presence of varying concentrations of inhibitor as compared to control was expressed as $\mu\text{mol S-2, 4-dinitrophenyl-GSH adduct formed/min} \pm \text{S.D.}$ based on experiments done in quadruplicates.

Central to the whole concept of chemotherapy is selectivity, the drug must affect the parasite, but not the host, although this can sometimes be very difficult to achieve. The continuing demand for new antiparasitic agents has always encouraged an ever-widening search for novel biochemical targets that can provide a basis for rational drug development. GST is therefore a tempting target both for chemotherapy and for vaccine development of filarial infections.

In general, helminth GST(s) are inhibited, as are mammalian GST(s), by a multitude of ligands from hematin to fatty acids. Helminth GST(s) appear to bind a range of hydrophobic ligands, including hematin related com-

pounds, bile acids, unsaturated fatty acids and plant phenols. Binding could also be a passive detoxification mechanism, as has been suggested for the interaction of hematin with *S. japonicum* GST (Smith *et al.*, 1986). This may indicate that, as proposed for a number of mammalian hepatic GST(s) (Ketterer *et al.*, 1988), helminth GST(s) are general binding proteins involved in the transport of hydrophobic ligands. Cestodes and digeneans have relatively high levels of GST, which provides high number of mobile hydrophobic binding sites that could aid the uptake of ligands required for intracellular metabolism. The inhibition of cestode GST(s) by ligands such as bile acids and unsaturated fatty acids suggests that these ligands may modulate the activity of the enzymes *in vivo*. Helminth GST(s) could also be intracellular receptors for bile acids. A variation in the inhibitor profile between human and parasitic GST(s) is more common than selective inhibition. For example, the major GST(s) from cestodes and *S. mansoni* are relatively more sensitive to inhibition to cibacron blue and triphenyltin chloride compared to the majority of mammalian GST(s) (Brophy and Barrett, 1990). Inhibition of OvGST2 has been demonstrated at low micro molar concentration for these conjugates and selectivity for OvGST over human pi GST of greater than 10-fold has been obtained (Brophy *et al.*, 2000).

The potential of GST as a drug target has previously been studied by studying the *in vitro* effects of some known GST inhibitors on the motility and viability of *S. cervi* adults and microfilariae (mf). Of all those tested, ethacrynic acid (EA), N-ethyl maleimide (NEM), 4-nitro-pyridine oxide and 1-chloro-2, 4-dinitrobenzene (CDNB) at micromolar concentrations reduced the viability and motility of mf, third stage larvae (L3) and adult worms. These results suggest that GST(s) are major metabolic enzymes and may play an important role in the parasite's survival (Rao *et al.*, 2000). Among the GST inhibitors studied, butylated hydroxyanisole (BHA) markedly reduced worm viability; compounds in this chemical series may have therapeutic use (Gupta & Rathaur, 2005).

If the present strategy i.e. inhibition of parasitic GST, affects the survival of the parasites or helps in the enhancement of activity of presently available antifilarial/antimalarial drugs, it will help in the design of new target based chemotherapeutic agents. The difference in the extent of inhibition of GST activities of the parasite and the host by the inhibitors tested in the present study indicates that these inhibitors have the potential to emerge as lead molecules for further exploration to develop new class of antifilarial/antimalarial drugs. Moreover, it has been suggested that perhaps it is not essential to target the parasite's enzymes specifically as it might be feasible simply to take advantage of the extreme sensitivity of parasite to oxidative damage (Lüersen *et al.*, 1998).

In future, pro-drug design could provide a route to parasite GST inhibition. This inhibitory mechanism could create selectivity by mimicking putative *in vivo* substrates of parasites GST(s), i.e. carbonyl products of immune-mediated lipid peroxidation. The conjugates of glutathione and

cytotoxic carbonyls are potent inhibitors of cestode and digenean GST(s). This will enhance our understanding of detoxification processes, drug resistance and drug action in the parasite and will open new avenues for the development of antiparasitic agents.

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