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# Filarial glutathione *S*-transferase: its induction by xenobiotics and potential as drug target

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**Glutathione-*S*-transferase (GST) a Phase-II drug detoxification enzyme, was detected in *Setaria cervi*, a bovine filarial parasite. *In vitro* effect of diethylcarbamazine, butylated hydroxyanisole and phenobarbitone on the GST of adult female *S. cervi* was assayed by the addition of these compounds in the maintenance medium. The specific activity of GST towards 1-chloro-2,4-dinitrobenzene was increased progressively 1.2–1.97, 1.3–2.4 and 1.2–2.7 times at 10–100 μM of diethylcarbamazine, butylated hydroxyanisole and phenobarbitone, respectively, after 5 h at 37ºC. Substrate specificity studies showed a higher increase in specific activity with ethacrynic acid and no change with cumene hydroperoxide. Although the intensity of GST activity band was more in extract from diethylcarbamazine or butylated hydroxyanisole treated worms extract, an extra band of activity appeared in those worm extracts compared to control worm extract. SDS/PAGE showed increased thickness of the band corresponding to purified GST in extracts from diethylcarbamazine/butylated hydroxyanisole/phenobarbitone treated worms. Purification and quantification of GST from diethylcarbamazine and butylated hydroxyanisole treated worms indicated an increase in enzyme specific activity. The increase in GST protein by these agents was blocked by prior treatment with actinomycin D, indicative of a transcription dependent response. The role of this enzyme in motility and viability of microfilariae and adult female was tested *in vitro* using a range of known GST inhibitors. Of those tested, ethacrynic acid, ellagic acid, 1-chloro-2,4-dinitrobenzene, cibacron blue and butylated hydroxyanisole reduced the viability and motility of microfilariae and adult female worms at micromolar concentrations. These results suggest that *S. cervi* GST is inducible in response to the antifilarial drug diethylcarbamazine and may play an important role in parasite's survival, thus could be a potential drug target.**

**Keywords:** lymphatic filariasis, *Setaria cervi*, glutathione-*S*-transferase, diethylcarbamazine, actinomycin D

Glutathione-S-transferase (GSTs, EC. 2.5.1.18) are multifunctional proteins that can function as enzymes catalyzing the conjugation of glutathione thiolate anion with a multitude of second substrates or as non-covalent binding proteins for a range of hydrophobic ligands (Mannervik & Danielson, 1988). Helminths have limited detoxification enzymes and appear to lack the important cytochrome P-450 dependent detoxification reaction (Precious & Barrett, 1989). GST has been detected in a range of helminths (Brophy & Barrett, 1990), where it may be one of the major detoxification enzymes, and probably plays a role in the survival of the parasites within the host environment. Drug resistance has been correlated to increased GST level in certain nematodes like *Haemonchus contortus* (Kwalek *et al.,* 1984). *Schistosoma mansoni* GST catalyzes the detoxification of dichlorvos, the active form of metrifonate, by O-demethylation (O'Leary & Tracy, 1991) and connection has also been found between salicylanilide resistance and GST activity in a number of isolates of *Fasciola hepatica*. The major GSTs isolated from the cestodes *Monienza expansa*, *Hymenolepis diminuta* and *Schistocephalus solidus* and the digenean *Fasciola hepatica* appear to bind a number of commercially available antihelminth-

**Abbreviations**: BHA, butylated hydroxyanisole; CB, Cibacron Blue; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; DEC, diethylcarbamazine; DMSO, dimethylsulphoxide; EA, ethacrynic acid; ElA, ellagic acid; GSH, glutathione; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NBT, nitroblue tetrazolium; PBS, phosphate-buffered saline; PhenoB, phenobarbitone; PMSF, phenylmethylsulfonyl fluoride.

ics and thereby detoxify them (Brophy *et al.*, 1989). So far, there have been no documented problems of antihelminthic resistance in lymphatic filaria. However, antihelminthic use is increasing and there is concern that resistance could become problematic in these human nematode parasites (Meredih & Dull, 1998; WHO Report 1998). Treatment of large human populations to break transmission of filarial nematodes and the tendency to increase the frequency of DEC treatment in lymphatic filariasis from once per year to two or more times per year will increase selection pressure for resistance (Prichard, 2001). Thus to counteract chemotherapeutic resistance, an understanding of the xenobiotic metabolism of these parasites is a prerequisite.

A range of chemical compounds that are able to selectively induce enzymes involved in detoxification reaction, e.g. compounds containing 1,2-dithiole motifs, simple aromatic compounds containing one or more N-hetero atoms and, phenolic anti-oxidants have been extensively investigated in rat (Pickett & Lu, 1989). Virtually nothing is known about induction of GST in nematode parasites. Because an increased capacity of the parasite to detoxicate xenobiotics could lead to decreased drug efficacy or even drug resistance, we sought to determine whether the level of GST in *Setaria cervi*, a bovine filarial parasite, is affected by exposure to the antifilarial drug DEC. For comparison prototypic inducing agents, such as butylated hydroxyanisole and phenobarbitone were used. These agents selectively induce drug detoxifying enzymes without having any effect on the cytochrome P-450 system. Induction is characterized by an increase in enzyme content or specific activity. Prior treatment with actinomycin-D is used to delineate induction responses that result from DNA-dependent mRNA synthesis.

## MATERIAL AND METHODS

**Collection of parasites and parasitic materials.** Adult *S. cervi* parasites were procured from the peritoneal folds of freshly slaughtered Indian water buffaloes. Worms were washed with phosphate- buffered saline (PBS) and maintained in Krebs' Ringer bicarbonate buffer supplemented with streptomycin, penicillin, glutamine and 1% glucose (maintenance medium).

Adult worm extract was prepared by homogenizing adult worms in 50 mM Tris/HCl buffer, pH 7.0, containing 1 mM PMSF and 1 mM EDTA using motor driven Remi type RQ127A homogenizer. The homogenate were centrifuged at 5000 × *g* for 20 min at 4ºC to remove the debris and the resulting supernatant was further centrifuged at 100 000 × *g* for 60 min at 4ºC. The 100 000 × *g* supernatants were collected, used for enzyme assay and purification. Protein concentration was estimated by the method of Bradford (1976) using bovine serum albumin as a standard.

**Exposure of worms to DEC, BHA and phenobarbitone**. Stock solutions of DEC, BHA and phenobarbitone (100 mM) were prepared in dimethylsulphoxide (DMSO) for further preparation of maintenance medium containing 10–100 μM of DEC, BHA and phenobarbitone. Equal numbers of adult female *S. cervi* were incubated in the medium containing 10–100 μM of DEC, BHA or phenobarbitone for 5 h at 37ºC. Worms incubated in the medium containing same amount of DMSO served as control. After each hour worms were transferred to fresh medium to maintain the pH and concentration of these xenobiotics. After 5 h worms were recovered and washed with PBS, homogenized and assayed for GST activity as described below. Each experiment was performed in duplicate with 810 different determinations. To study the mechanism of induction actinomycin D was added to the medium 1 h prior to the addition of inducing agents (100 μM). This experiment was carried out in duplicate with three different determinations.

To see the effect of DEC and BHA on the GST of microfilariae of *S. cervi*, microfilariae were incubated in maintenance medium containing 50 μM DEC or 100 μM BHA for 4 h at 37ºC. Microfilariae incubated in the maintenance medium only served as control. After 4 h the medium was centrifuged for 10 min at 2 000 r.p.m. at 4ºC to settle the microfilariae. Supernatant was removed and pellet was washed twice with PBS. The soluble extract of mf was prepared by sonicating the microfilariae using Remi ultrasonic disintegrator for 10 min, with cycle of 30 s sonication followed by 30 s interval at 20 kHz in cold 20 mM phosphate buffer, pH 7.0, containing 1 mM EDTA and 1 mM PMSF. The extract was centrifuged at 16 000 × *g* for 30 min. The clear supernatant was treated as microfilariae extract and stored at –70ºC until used. GST activity in the extract was measured using 1-chloro-2,4-dinitrobenzene as substrate.

**Enzyme assay.** GST activity in worm homogenates was measured spectrophotometrically at 340 nm with Perkin Elmer double beam spectrophotometer according to the method of Habig *et al.* (1974), using CDNB and GSH as substrates. The reaction mixture contained 25 μl of extract, 1 mM GSH and 1 mM CDNB and assay was conducted at 25ºC in 0.1 M phosphate buffer, pH 6.5. Substrate specificity of GST in control and treated worm extract was studied using ethacrynic acid, 1,2-dichloro-4-nitrobenzene (DCNB) and bromosulphophthalein as described by Habig *et al.* (1974). Activity with cumene hydroperoxide as substrate was determined according to the method of Pagalia and Valentine (1976).

**Enzyme activity staining**. Soluble extract from DEC or BHA treated and control *S. cervi* containing equal amount of protein were run on 10% native PAGE according to the method of Laemmli (1970) and stained for GST activity using the method of Ricci *et al.* (1984). Briefly, after electrophoretic run, the gel was equilibrated in 0.1 M potassium-phosphate buffer, pH 6.5, for 10 min, and transferred to a reaction mixture containing 4.5 mM GSH, 1 mM CDNB and 1 mM nitroblue tetrazolium in 0.1 M potassium-phosphate buffer, pH 6.5, at 37°C for 10 min. Further the gel was incubated at room temperature in 0.1 M Tris/HCl, pH 9.6, containing 3 mM phenazine methosulphate. The activity band appeared as an achromatic zone against a blue background. Similarly, extracts containing equal amount of protein or purified *S. cervi* GST were run on 10% SDS/PAGE and stained for protein with Coomassie blue stain.

**GST purification**. Soluble extracts containing equal amount of protein were loaded separately on an affinity column and GST was purified (Simons & Vander Jagt, 1977). In brief, 100 000 × *g* supernatant of adult *S. cervi* was applied on GSH-agarose (Sigma) affinity column (1 × 1 cm) pre-equilibrated with 20 mM phosphate buffer, pH 7.0, containing 0.1% triton X-100, 0.1 mM PMSF and 1 mM EDTA at 4°C. Unbound proteins were washed through the column with six-bed volume of the above buffer at the flow rate of 15 ml $h^{-1}$. Non-specifically bound proteins were eluted with 50 mM Tris/HCl buffer, pH 9.6, containing 1 mM EDTA, 0.1 mM PMSF (elution buffer). GST activity (bound protein) was eluted from the column with elution buffer containing 15 mM GSH. Fractions (1 ml) were collected and monitored for protein at 280 nm and GST activity at 340 nm as described above. Fractions having GST activity were neutralized, pooled, concentrated, dialyzed against 50 mM Tris/HCl, pH 7.0, and stored at –20°C.

**Effect of inhibitors on parasite viability**. Adult female worms (n = 6) were incubated either in medium alone or in medium containing inhibitors (CDNB, EA, ElA, CB, BHA, ascorbic, palmitic and stearic acid) at appropriate concentrations under sterile condition at 37°C for 6–24 h. Different concentrations of these inhibitors in soluble form were prepared fresh before they were added to the medium. The pH of the working solution containing the inhibitor was adjusted to 7.5. Frozen parasites were thawed and used as negative controls. Experiments were carried out in triplicate and the results were expressed as an average of two independent experimental values. Parasite motility was assessed visually using a compound light microscope. Parasite viability was assessed quantitatively by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) reduction assay. The procedure used was as described by Rao *et al.* (1991). Briefly, after microscopic assessment of parasite viability, adult worms were transferred to 5 ml and microfilariae to 0.1 ml of PBS containing 0.5 mg MTT $ml^{-1}$, and were incubated for 2 h at 37°C in the dark. DMSO was added and incubated for 1 h to dissolve the dark blue crystals of formazan and the mixture was then transferred to 96 well microtitre plate. Formazan quantitation was performed with microplate reader (BioRad, 550) at 540 nm, using DMSO as a blank. The absorbance values, relative to those for the controls, were compared for differences in inhibition of viability. Greater than 50% inhibition in MTT reduction was considered significant. $IC_{50}$ values for each inhibitor were obtained graphically.

**Statistical analysis**. Mean, standard deviation and statistical significance were calculated using the PC SSPS application package. Statistical significance between the worms that were treated with DEC, BHA or phenobarbitone and the control worms was determined by using a one-way Student's *t*-test and $P < 0.005$ was considered significant.

## RESULTS

### *In vivo* effect of DEC, BHA and phenobarbitone on the GST activity of *S. cervi*

A significant increase in the specific activity of GST was observed in adult female *S. cervi* after *in vitro* exposure to DEC, BHA or phenobarbitone. The specific activity of GST towards 1-chloro-2,4-dinitrobenzene was increased progressively 1.2–1.97, 1.3–2.4 and 1.2–2.7 times in adult female worms recovered from the maintenance medium containing 10–100 μM of DEC, BHA and phenobarbitone, respectively, after 5 h at 37°C (Fig. 1).

#### Substrate specificity

The substrate specificity of GST in control and DEC or BHA treated worms showed that the increase in specific activity of GST was higher with ethacrynic acid in comparison to CDNB. However, the activity was not detectable when DCNB or bromosulfophthalein were used as substrate. The specific activity with cumene hydroperoxide did not change following DEC and BHA treatment (Table 1).

#### Effect of xenobiotics on worms motility and viability

DEC and phenobarbitone had no effect on the viability/motility of *S. cervi* even at higher concentration within the observation period. However, BHA has marked effect on the motility and viability depending upon the concentration. At the concentration 100–200 μM of BHA worms became immobile

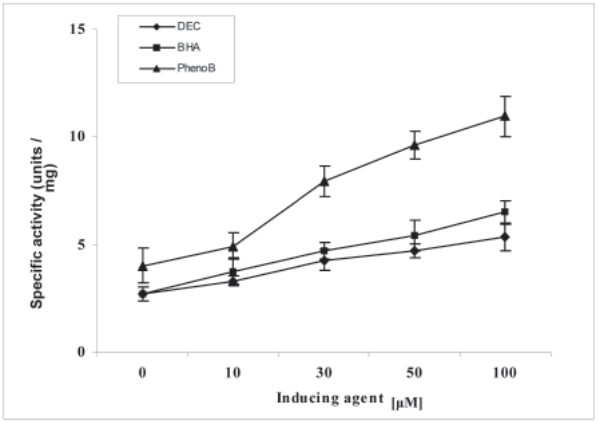


**Figure 1.** *In vivo* **effect of DEC (♦), BHA (■) and phenobarbitone (▲) on GST of adult female** *S. cervi.*

Specific activity of GST was determined in adult female *S. cervi*, recovered from medium containing 0, 10–100 μM of DEC/ BHA/phenobarbitone (PhenoB) after 5 h at 37°C. One unit of enzyme activity is defined as the amount of enzyme required to convert 1 μmol of substrate (CDNB) into product in 1 min per ml reaction mixture under assay conditions. Specific activity is units of enzyme per mg of protein. Values are expressed as mean ± S.D., n = 8–10 determinations.

within 10–20 min but they revived when transferred to fresh medium. At concentrations above 200 μM BHA death of the parasites was observed within 5–10 min. About 50–60% microfilariae obtained after dissecting these dead worms were also found dead.

### GST activity staining and SDS/PAGE analysis

Extracts from control, DEC and BHA treated worms containing equal amount of protein were run on 10% non-denaturing polyacrylamide gel and stained for GST activity. A doublet of GST activity bands was observed, with a higher intensity in DEC and BHA treated worm extracts as compared to control worm extract. An extra band of GST activity was observed in extracts from BHA and DEC treated worms (Fig. 2A, B). The intensity of the extra band was higher in BHA treated than DEC treated worms. A similar pattern of activity bands was observed when ethacrynic acid was used as substrate. To see whether this increase in enzyme activity is due to activation of GSTs or expression of more GST protein, extracts containing equal amount of protein were run on 10% SDS-gel and stained for protein. Purified GST was run in the same gel for comparison. As shown in Fig. 2c, a slight increase in the density of the protein band corresponding to purified GST in extracts from DEC, BHA and phenobarbitone treated worms compared to control was observed. This increase in protein content was further confirmed by purification.

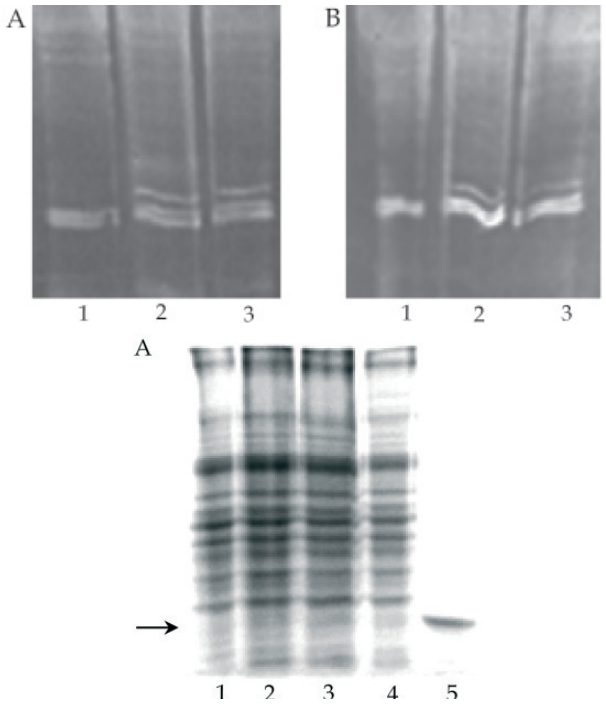


**Figure 2. Enzyme staining of GST in 10% native gel using CDNB (A), or ethacrynic acid (B) as substrate.**

After running the extract in native PAGE, GST activity staining was done according to Ricci *et al.* (1984) by incubating gel in reaction mixture containing 4.5 mM GSH, 1 mM CDNB and 1 mM NBT in 0.1 M phosphate buffer, pH 6.5, at 37°C for 10 min and activity bands were visualized by incubating at room temperature in 0.1 M Tris/ HCl, pH 9.6, containing 3 mM PMS. lane 1, control; lane 2, BHA treated; lane 3, DEC treated. SDS/PAGE analysis and Coomassie staining (C) of extracts from control and treated *S. cervi.* Lane 1, control; lane 2, BHA treated; lane 3, DEC treated; lane 4, phenobarbitone treated; lane 5, affinity purified *S. cervi* GST.

### Purification

GST was purified from 100 000 × *g* supernatant of adult *S. cervi* by affinity chromatography on GSH-agarose column, with a similar elution pattern for control, DEC and BHA treated worms. GST containing fractions from DEC and BHA treated worms showed a significant increase in activity. A portion of GST activity did not bind to the GSH-agarose affinity column in all the samples. The increase in specific activity of purified GST was 2.0 and 2.4 times in DEC and BHA (Fig. 3) treated worms, respectively, as compared to control. The increase in GST protein was 30% and 34% in those worms, respectively.

This increased GST protein content after exposure to DEC and BHA could result either from transcriptional activation of the subunit gene or from stabilization of the message (mRNA) itself. Therefore to delineate induction responses that result from DNA-dependent mRNA synthesis actino-

**Table 1. Substrate specificity of GST in control, DEC and BHA treated adult female *S. cervi*.**

| Inducing agents (μM) | Specific activity (μM $min^{-1}$ $mg^{-1}$) | | | | |
|---|---|---|---|---|---|
| | CDNB | Bromosulphophthalein | Ethacrynic acid | DCNB | Cumene hydroperoxide |
| None | 3.23 ± 0.52 | ND | 4.21 ± 0.21 | ND | 2.51 ± 0.13 |
| DEC | 6.14 ± 0.61* | ND | 10.43 ± 0.15* | ND | 2.42 ± 0.24 |
| BHA | 7.43 ± 0.43* | ND | 12.63 ± 0.51* | ND | – |

Values are expressed as mean ± S.D.; n = 3 determinations. ND, not detectable; *Significantly different ($P < 0.005$) from control.

mycin D was used. Worms were treated with 2 mg/ml of actinomycin D for 1 h prior to the exposure to inducing agents. No increase in GST specific activity was observed in worms treated with actinomycin D 1 h prior to the addition of inducing agent (100 μM) (Table 2).

### *In vivo* effect of DEC and BHA on the GST activity of microfilariae of *S. cervi*

The specific activity of GST towards 1-chloro-2,4-dinitrobenzene was increased 2.6 times in microfilariae recovered from the maintenance medium containing 100 μM of BHA after 5 h at 37°C. However, a decrease in GST specific activity was observed in worms treated with 50 μM DEC (Table 3) and the activity was not detectable in microfilariae treated with 100 μM DEC.

### Effect of inhibitors on parasite motility and viability

To determine whether GSTs have any role in the motility and viability of *S. cervi*, the effect of a battery of known GST inhibitors of mammalian GST was tested on the viability of female worms and microfilariae. Adult females and microfilariae were incubated separately *in vitro* with various inhibitors for 24 h. The motility of adult female worms was determined by eye and of microfilariae microscopically and viability was measured by MTT reduction assay. At concentrations of 0.05–0.3 mM GST inhibitors such as ascorbic, stearic and palmitic acid showed no effect on parasite motility and viability. However, at those concentrations, ethacrynic acid, ellagic acid, BHA, cibacron and CDNB individually reduced the motility of adult worm and microfilariae by 10 h and viability by 24 h and the parasites were not revived when transferred to fresh medium. The individual effect of ethacrynic acid, ellagic acid, Cibacron Blue and CDNB on viability of microfilariae was further tested by determining the ability of the parasites to reduce the tetrazolium salt MTT to its formazan product. At 24 h these inhibitors reduced formazan formation. In contrast, microfilariae cultured without inhibitors actively incorporated MTT and reduced it to purple formazan. The viability of adult worms and microfilariae was decreased by 50% or more with these inhibitors at a concentration greater than 0.01 mM and above. The $IC_{50}$ values of inhibitors are shown in Table 4.

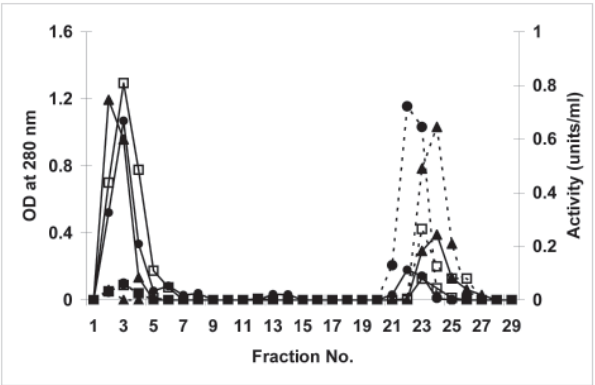


**Figure 3. GSH-agarose affinity purification profiles of GST from *S. cervi* control (❑), BHA (▲) and DEC treated (●).**

Equivalent amount of protein were applied to each column, unbound fraction washed through with the 20 mM potassium-phosphate buffer pH 7.0, non-specific bound protein was washed with 50 mM Tris/HCl, pH 9.6, and bound protein (GST) eluted with 15 mM GSH in 50 mM Tris/HCl buffer, pH 9.6. Fractions (1 ml) were collected and monitored for protein (—) and GST activity (-----).

## DISCUSSION

Significant activity of GST was detected in *S. cervi.* GST is the main detoxifying enzyme in parasites and has been correlated to drug resistance, therefore the present work was planned to see the effect of some xenobiotics like the antifilarial drug

**Table 2. Induction of *S. cervi* GST by DEC, BHA and phenobarbitone**

| Inducing agents | Specific activity (μM $min^{-1}$ $mg^{-1}$) | Fold induction |
|---|---|---|
| None | 2.64 ± 0.13 | – |
| ActinoD+DEC (100 μM) | 2.44 ± 0.16 | 0.924 |
| DEC (100 μM) | 5.2 ± 0.26* | 1.97 |
| ActinoD+BHA (100 μM) | 2.61 ± 0.16 | 0.99 |
| BHA (100 μM) | 6.08 ± 0.32* | 2.3 |
| ActinoD+PhenoB (100 μM) | 2.56 ± 0.12 | 0.97 |
| PhenoB (100 μM) | 7.36 ± 0.46* | 2.79 |

Values are expressed as mean ± S.D., n = 3–4; ActinoD, actinomycin D, 2 mg/ml given 1 h before the inducer. PhenoB, phenobarbitone; *Significantly different ($P < 0.005$) from control.

**Table 3. *In vivo* effect of DEC and BHA on GST activity of microfilariae of *S. cervi*.**

| Inducing agent | Activity (mM $min^{-1}$ $ml^{-1}$) | Protein (mg/ml) | Specific activity (mM $min^{-1}$ $mg^{-1}$) |
|---|---|---|---|
| None | 0.042 ± 0.01 | 0.8 ± 0.12 | 0.052 ± 0.1 |
| DEC (50 μM) | 0.021 ± 0.008 | 1.0 ± 0.25 | 0.011 ± 0.01* |
| BHA (100 μM) | 0.083 ± 0.021 | 0.6 ± 0.2 | 0.139 ± 0.05* |

Values are mean ± S.D. of three determinations; *Significantly different ($P < 0.05$) from control.

DEC, the phenolic antioxidant BHA and phenobarbitone on the GST of *S. cervi*. In this study we have demonstrated that *S. cervi* GST can be induced by DEC, BHA and phenobarbitone. Although GSTs have been shown to be inducible in various animal and plant species (Clark, 1989), little is known about the effect of xenobiotics on GST expression in parasites of medical and veterinary importance. There have been reports that GST activity can be induced by phenobarbitone in the cestode *Echinococcus granulosus* (Morello *et al.,* 1982), and in *H. diminuta* (Brophy & Barrett, 1990) and by BHA and phenobarbitone in *S. mansoni* (Vandewaa *et al.,* 1993).

*In vitro* maintenance of adult female *S. cervi* in medium containing BHA, phenobarbitone and the antifilarial drug DEC resulted in a significant increase in GST specific activity. This induction of GST was dose and time dependent. The substrate specificity of GST in these extracts showed a higher increase in specific activity with ethacrynic acid in comparison to CDNB suggesting that the induced enzyme is related to the Pi class of mammalian GST (Mannervik *et al.,* 1988). The results suggest that DEC affects the expression of *S. cervi* GST.

DEC and phenobarbitone has no effect on the viability of *S. cervi* even at a higher concentration within the observation period. However, BHA was found to have a marked effect on the motility and viability of parasites at a high concentration. The mechanism by which BHA affects the parasite survival is not clear. Jaiswal (2004) has reported that antioxidants and xenobiotics are metabolized by cellular enzymes to generate superoxide and electrophiles. It is believed that this initial generation of superoxide activates a battery of genes for cellular protection. Failure of this mechanism leads to the accumulation of superoxide and other free radicals, which in turn causes oxidative stress, membrane damage, degeneration of tissues and apoptotic cell death (Rushmore *et al.,* 1990; Riley & Workman, 1992; Tsuchida & Sato, 1992; Daniel, 1993; Rushmore & Pickett, 1993; Talalay *et al.,* 1995). The effect of BHA at a high concentration on worm viability, and its ability to induce *S. cervi* GST significantly at a low concentration differentiate *S. cervi* GST from the mammalian enzyme, which requires very high doses of BHA for significant induction (Strohmayer *et al.,* 1980).

**Table 4. *In vitro* effect of GST inhibitors on motility and viability of adult female and microfilariae of *S. cervi*.**

| Inhibitor | $IC_{50}$* (mM) | |
|---|---|---|
| | Adult female | Microfilariae |
| Ethacrynic acid | 0.02 | 0.038 |
| Ellagic acid | 0.021 | 0.046 |
| Cibacron Blue | 0.05 | 0.025 |
| CDNB | 0.032 | 0.015 |
| BHA | 0.07 | 0.05 |
| Palmitic acid | – | – |
| Stearic acid | – | – |
| Ascorbic acid | – | – |

*Values are the mean of duplicate samples of three independent experiments. The standard deviation between duplicate samples was less than 10% and did not vary with concentration. The percentage of viability inhibition was calculated from MTT reduction assay.

The activity staining on gel using ethacrynic acid and CDNB as substrate showed an increase in the intensity of GST activity band in extracts from DEC and BHA treated compared to control worms. The appearance of an extra band in DEC and BHA treated worm extracts suggests that this form of GST is stress responsive. A stress responsive form of GST has also been reported in *O. volvulus* (Liebau *et al.,* 2000) and related to the Pi class. This increase in enzyme activity may result either from activation of the enzyme or from a higher expression of the GST protein. SDS/PAGE analysis of control and treated worm extract showed a slight increase in the thickness/density of protein bands corresponding to GST in extracts from DEC, BHA and phenobarbitone treated worms. This increase in GST protein was further confirmed by purification of GST from these extracts using GSH-agarose affinity column.

Purification of GST from the treated worms suggests that the enzyme was still capable of binding to GSH-agarose and showed a similar pattern of elution as control, showing no structural change. The increase in the GST protein content after DEC and BHA treatment was significant, but did not appear to be proportional to the increase in activity. This suggests that the GST produced during stress was more active. The GST induction by BHA and phenobarbitone was similar to that observed for *S. mansoni* GST (Vandewaa *et al.,* 1993). The increased GST protein content after exposure to DEC and BHA could result either from transcriptional activation of the sub-unit gene or from stabilization of the mRNA itself. The increase in GST protein by these agents

was blocked by prior treatment with actinomycin D, indicative of a transcription-dependent response. In *S. mansoni*, induction of GST by xenobiotics has also been shown to be transcriptionally mediated (Vandewaa *et al.*, 1993).

The investigated N-heterocyclic compound diethylcarbamazine, although it lacks the 1,2-dithiole motif, is effective in inducing detoxification enzymes and acts through a transcriptional response. At what stage the transcriptional response coincides with the mechanism for other selective inducers of detoxifying enzymes is not known. It is unlikely to be coincident with the initial steps for oltipraz induction where the formation of dithiols with proteins containing vicinal thiols has been implicated (Kensler *et al.*, 1999). Whether it is the same final step as for phenolic antioxidants induction where the Nrf2-MafK heterodimer interaction with gene regulatory elements is implicated (Itoh *et al.*, 1997) is unknown.

The effect of DEC on GST of microfilariae of *S. cervi* was found to be different from that on adult parasite. Unlike in adult parasite, the specific activity of GST decreased in microfilariae recovered from medium containing 50 μM DEC after 5 h at 37ºC. However, an increase in GST specific activity was observed in microfilariae recovered from medium containing 100 μM BHA. Thus DEC has different effects on GST of adult and microfilariae of *S. cervi.*

The antifilarial drug DEC is microfilaricidal and has been shown to have partial or no effect on adult parasites. One can speculate that the microfilaricidal activity of DEC partially depends on the inhibition of GST in microfilariae. Since in adult female *S. cervi* GST is inducible by DEC, this might be one of the bases of infectiveness of DEC on adult parasites.

We also studied the potential of GST as a drug target by studying the *in vitro* effects of some known GST inhibitors on the motility and viability of *S. cervi* adult and microfilariae. Ethacrynic acid, ellagic acid, CDNB, BHA and Cibacron Blue significantly reduced the viability and motility of the parasite suggesting that GST plays an important role in parasite survival. Among the GST inhibitors studied BHA markedly reduced worm viability; compounds in this chemical series may have therapeutic use.

GSTs as well as other GSH dependent enzymes have been implicated in protection of the parasite against oxidative stress (Tracy *et al.*, 1983). These observations together with the potential that GST could be induced by exposure of the parasite to drugs and other xenobiotics or to reactive oxygen species pose a number of questions about the biological and chemotherapeutic ramification of elevated GST in filarial parasites. Further, it may be hypothesized that inducible parasite specific GSTs contribute to chemotherapeutic efficacy, drug concentration, metabolism and resistance. Intervention of this enzyme could lead to an innovative approach for parasite control.

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