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Comparative Glutathione S-Transferases (GSTs) Inhibition Assay in the Whole Extract of *Fasciola hepatica* and Sheep Liver Tissue by Hexachlorophene

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ABSTRACT

Recent research highlights the importance of GSTs in the establishment of chronic helminth infections. GSTs have the potential to protect the parasite against the host immune response. In this present study, GST enzyme assay has been investigated on whole extract of *F. hepatica* and sheep liver tissue. To the 1-ml plastic cuvette, added 200 mM potassium phosphate buffer. Then added 50 Mm GSH reduced. Was placed the required volume of *F. hepatica* or sheep liver extract into the cuvette and mixed well. Added water and equilibrated at room temperature for 5 minutes. Meanwhile was set up the UV spectrophotometer at 340 nm for the GST assay. Finally was placed the cuvette into the barrel of the UV spectrophotometer and added 1-chloro-2, 4-dinitorbenzene (CDNB) and stirred well. For testing an inhibitor of GSTs such as hexachlorophene, mixed the appropriate volume of compound with GSH,reduced buffer, water and protein before equilibrating at room temperature. For calculation of $IC_{50\%}$ value a computer package was used. The inhibitor concentration for 50% remaining activity of GSTs calculated graphically and wasobtained 10µl for *F. hepatica* and 20 µl for liver tissue.

INTRODUCTION

GSTs have been extensively investigated in parasitic worms with respect to their biochemistry and independently GSTs have been identified as potential vaccine candidates in digenean parasites. Recent research highlights the importance of GSTs in the establishment of chronic helminth infections. GST appears to be the major phase II detoxification system present in parasitic worms and there is no evidence that the oxygen dependent P-450 system (usually the major Phase I detoxification mechanism in organisms) is expressed in adults. General biological roles of helminth GSTs include xenobiotic detoxification and ligand binding/transport functions. Moreover, the ability of helminth GSTs to effectively neutralise known cytotoxic products arising from reactive oxygen species (ROS) attack on cell membranes provides evidence that GSTs have the potential to protect the parasite against the host immune response (8). The immune-defence theory indicates that parasites express a range of anti-oxidant to counteract the host reactive oxygen species directed immune-effector system. To date several glutathione dependent components of the parasite defence system has been characterised, including glutathione S-transferases (GSTs), glyoxylase and glutathione reductase.

Thiol transferases (glutaredoxin) catalyses the reduction of disulfied bonds of various protein (and non-proteins) in the presence of glutathione (GSH), and one role proposed for the mammalian enzyme is to regenerate oxidatively damaged proteins. Thus the hypothesis is that parasitic helminths express high levels of thiol transferase as a part of their immune defence system (Brophy:personal communication). Investigation of parasite GSTs would link parasite immunology and biochemistry research. This hypothesis has been reviewed and is based on the supposition of immuno-and/or chemotherapeutic neutralisation of parasite immune defences (4). The soluble forms of GST would appear to be dimmers, and the molecular mass of GST subunits varies between 23 and 26 kDa. GST has been found in all adult helminths analysed for activity (1). In *Fasciola hepatica* GST is associated with the lamellae of the intestinal epithelium. GST may have a wide distribution in *F. hepatica* compared to *S. mansoni* . In *F. hepatica* GSTs are found in the tegument, muscular tissues,parenchymal cells, and the intestine (9).

GST as on Immunotherapeutic Target

The role of GST as a protective antigen in vaccination against digeneans is well documented. The Sm28 GST derived from *S.mansoni* has produced significant protection against schistosomiasis in animal hosts such as rat, mouse, baboon and hamster (5). Native GSTs from *F. hepatica* plus adjuvant provided significant protection against liver fluke infection in sheep. Hosts with fascioliasis can either be responders (rabbit and sheep) or nonresponders (mice and cattle) with respect to antibody responses to *F. hepatica* GST (7). GST antigen was on average four fold less effective in *F. gigantica* than in *F. hepatica* and this is because probably six fold less overall GST activity in *F. gigantica* (6).

GST as a Chemotherapeutic Target

In general, helminth GSTs are inhibited, as are mammalian GSTs, by a multitude of ligands from haematin to fatty acids. A variation in the inhibitor profile between human and parasite GSTs is more common than selective inhibition. For example, the major GSTs from cestodes and *S. Mansoni* are relatively more sensitive to inhibition to cibacron blue and triphenyltin chloride compared to the majority of mammalian GSTs (1). Inhibition of OvGST2 has been demonstrated at low micro molar concentration for these conjugates and selectivity for OvGST over human πGST of greater than 10-fold has been obtained (2).

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In the present study, GST inhibition enzyme assay has been investigated on whole extract of *F. hepatica* and sheep liver tissue by hexachlorophene.

MATERIALS AND METHODS

Sheep liver tissue and *Fasciola hepatica* helminths were collected from Aberystwyth abattoir, Wales, UK. *F. hepatica* species identified, based on morphological characters. After tissue homogenising to a 1-ml plastic cuvette, added 500µl of the 200 mM potassium phosphate buffer (final concentration of 1mM). Then 20µl of 50 mM GSH was added (final concentration of 1mM). Then the required volume of *F. hepatica* or sheep liver extract was placed into the cuvette and mixed well. Water was added to a volume of 980µl; equilibrated at room temperature for 5 minutes. Meanwhile, the UV spec was set up at 340 nm for the GST assay, also making sure that the assay was set for a kinetic run. Finally the cuvette was placed into the barrel of the UV spectrophotometer and added 20µM of CDNB and stirred well (final concentration of 1mM) and pressed start on the UV spectrophotometer machine.

For testing an inhibitor of GST such as hexachlorophene, the appropriate volume of compound was mixed with GSH, reduced buffer, water and protein (whole extract) before equilibrating at room temperature. For calculation of $IC_{50\%}$ value, a computer package was used.

RESULTS AND DISCUSSION

The average and standard deviation of remaining activity of *F. hepatica* and sheep liver tissue GSTs were obtained as follow (Table 1,2).

The inhibitor concentration for 50% remaining activity of *F. hepatica* GSTs was calculated graphically and was 10 µM (Fig.1).

The inhibitor concentration for 50%remaining activity of sheep liver tissue GST scalculated graphically and was obtained 20 µM (Fig. 2).

The hexachlorophene showed inhibitory activity against *F. hepatica* and host liver tissue GSTs. Comparison of the effect of hexachlorophene revealed that the activities of both GSTs were suppressed and the difference between the extent of inhibition was relatively high. This general inhibition of helminth and liver tissue GSTs in the micromolar range (as judged by $IC_{50\%}$ value) by antihelmintics may help explain the mode of action of this chemotherapeutic agents. A series of ß-carbonyl substituted glutathione conjugates have been evaluated as inhibitors of OvGST2 (2). However the binding of antihelmintics by a helminth GSTs may contribute to a passive detoxification mechanism (3). Purified GSTs assay is under study.

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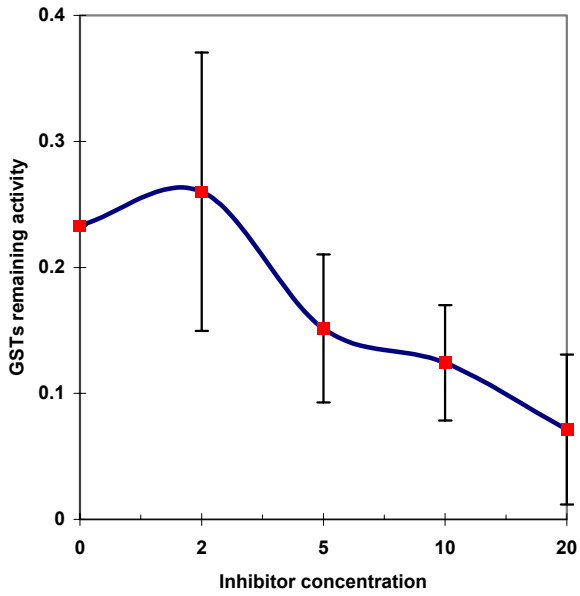
Table 1. **Average and standard deviation of GSTs remaining activity (mM per minute) based on inhibitor concentration**

Concentration of inhibitor (µl)	0	2	5	10	20
St.deviation	0.110431	0.058726	0.045764	0.059464	0.029529
Average	0.2331	0.260133	0.151467	0.124233	0.071333

Table 2. **Average and standard deviation of GSTs remaining activity (mM per minute) based on inhibitor concentration**

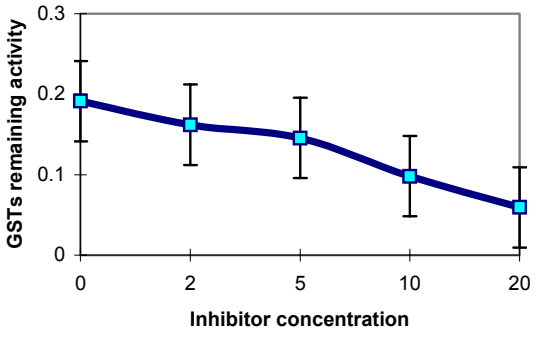
Concentration of inhibitor (µM)	0	2	5	10	20
S.deviation of GSTs activity	0.004005	0.014987	0.006592	0.011666	0.003342
Average of GSTs activity	0.191433	0.161967	0.145633	0.098167	0.0593

Fig. 1. **GSTs inhibition assay of *Fasciola hepatica* by various concentration of hexachlorophene**



$IC_{50\%}$ value = 10 µM

Fig. 2. **GSTs Inhibition assay of sheep liver tissue by various concentration of hexachlorophene**



$IC_{50\%}$ value = 20 µM

REFERENCES

Brophy PM, Barrett J (1990): Glutathione transferase helminths.*Parasitology,* **100**:345-9.

Brophy PM, Campbell AM, Van Eldick AJ, Teesdale-Spittle PH, Liebau Wang MF (2000): ß-Carbonyl substituted glutathione conjugates as inhibit of O. volvulus GST2. *Bioorg Med Chem Lett,* **10**:979-81.

Brophy PM, Crowley P, Barrett J (1990): Detoxification reaction of *Fasci hepatica* cytosolic glutathione transferases. *Mol Bioch Parasitol,* **39**:155-6

Brophy PM, Pritchard DI (1992b): Immunity to helminths-ready to tip biochemical balance. *Parasitology Today* **8**:419-22.

Brophy PM, Pritchard DI (1994): Parasitic helminth glutathione transferases: An update on their potential as targets for immuno-a chemotherapy. *Exp Parasitol* **79**:89-96.

6. el-Ghayash AA, Barrett J, Brophy PM (1999): Expression of glutathione S-transferases (GSTs) in *F. gigantica* derived from three host (cattle, water buffaloes and donkeys). *Helminthologia* **36**:5-8.7.
7. Hilyer GV, de Galanes MS and Battisti G(1992):*Fasciola hepatica:* Host responder and nonresponders to parasite glutathione S-transferase. *Exp Parasitol,* **75**:176-86.
8. Hompage of Parasitology, Aberystwyth University of Wales Institute of Biological Sciences, Parasitology Research Group: (http://www.aber.ac.uk/~mpg /www).
9. Panaccio M, Wilson LR, Crameri SL, Wijffels GL, Spithill TW (1992): Molecular characterisation of cDNA sequences encoding glutathione S-transferases of *Fasciola hepatica. Exp Parasitol*, **74**:232-7.