INHIBITION ASSAY STUDY OF PURIFIED GLUTATHIONE S-TRANSFERASE FROM *FASCIOLA HEPATICA* AND SHEEP LIVER TISSUE BY HEXACHLOROPHENE

A. Farahnak[1]* and PM. Brophy[2]

1) Department of Parasitology and Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2) Department of Biological Sciences, University of Wales, Aberystwyth, Wales, UK

Abstract- Glutathione S-transferases (GSTs) are widespread in *Fasciola. hepatica* parasite and sheep liver tissue. Study of GSTs inhibition assays in *F. hepatica* and sheep liver tissue are a priority of chemotherapeutic targets in parasitic liver diseases including human fascioliasis in Iran. In this research, the whole extract of *F. hepatica* and sheep liver tissues were purified and eluted for sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) pattern and GSTs inhibition assay. GSTs inhibition was detected by hexachlorophene as an inhibitor and 1-chloro-2,4-dinitrobenzene (CDNB) as secondary substrate. The purified GSTs from *F. hepatica* and liver tissue contained comparable components and showed a molecular weight of 26kDa. The inhibitor concentration of hexachlorophene, for the remaining 50% activity (IC50%) of GST enzymes from *F. hepatica* and liver were graphically calculated, and the results were 0.25 µM and 1 µM, respectively. GSTs of *F. hepatica* may be more sensitive than sheep liver tissue to hexachlorophene.

Acta Medica Iranica, 42(3): 168-171; 2004

Key words: Glutathione S-transferase, *Fasciola hepatica*, purification, inhibition assay

INTRODUCTION

Fasciola hepatica is the causative organism of fascioliasis. A wide variety of mammals, including man may be infected with *F. hepatica* by ingestion of contaminated vegetables such as watercress. Glutathione transferase is one of the major detoxification systems found in helminths, including *F. hepatica* (1). Glutathione S-transferases (GSTs) are found in high levels in *F. hepatica*; the level of this enzyme is approximately 4% of the total soluble protein (2, 3). The helminth GSTs are present as isoenzymes but fail to show a clear biochemical homology to any of the three mammalian GST families. GSTs bind to a range of antihelmintics but there is limited evidence that the enzymes can conjugate anthelmintics with glutathione (4). Acidic and neutral GST forms have been isolated from *F. hepatica* by a combination of glutathione affinity chromatography and chromatofocusing (5). *F. hepatica* GSTs were isolated from adult worms by glutathione agarose affinity chromatography. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) shows three proteins of M(r) ranging from 29-27.8 kDa. (6). Four cDNAs encoding GST (rGST1, rGST7, rGST47 and rGST51) of *F. hepatica* have been expressed in *Escherichia coli*. The rGST proteins were 95% pure as indicated by Coomassie staining of proteins separated by SDS-PAGE. All four rGST proteins from *F. hepatica* actively conjugate glutathione with the universal substrate, 1-chloro-2,4-dinitrobenzene (7). Studies on GST enzymes in *F. hepatica* as a parasite, and sheep liver tissue as a host, are the priority of chemotherapeutic and immunotherapeutic targets in fascioliasis.

Received: 27 Apr. 2002, Revised: 30 Jun. 2003, Accepted: 15 Oct. 2003

*** Corresponding Author:**
A. Farahnak, Department of Parasitology and Mycology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 8951583, Fax: +98 21 6462267
E-mail: farahnak@sina.tums.ac.ir

In the present research, the whole extracts of *F. hepatica* and sheep liver tissue were purified, and eluted solutions were investigated for molecular weight by SDS-PAGE, and GSTs inhibition assay by hexachlorophene as an inhibitor.

MATERIALS AND METHODS

GSTs purification

GSTs were purified from adult *F. hepatica* and sheep liver tissue as an enzyme pool by a glutathione affinity matrix using a wash-bath method (4). Approximately 200 µl wet volume of freshly prepared glutathione-agarose gel (Sigma) was washed with 1000 µl of 20 mM potassium phosphate buffer (pH 7.0) containing 50 mM sodium chloride (solution A) in a micro centrifuge tube (1500 µl capacity) by centrifugation at low speed (9000 rpm) for 10 sec. The extract, 1000 µl (3 mg protein for *F. hepatica* and 3 mg protein for liver tissue), was mixed with the gel for 30 min at 4°C. The supernatant was removed by centrifugation and the gel matrix washed with 1000lµl of solution A. Polypeptides were eluted from the gel by washing with 5 × 200 µl of 50 mM Tris-HCL pH 9.6 buffer (4°C) containing 5 mM glutathione (reduced). Concentration of solubilized protein of *F.hepatica* and liver tissue, before and after purification was determined by Bio-Rad protein assay, based on the method of Bradford .

SDS-PAGE pattern of GSTs

For SDS-PAGE whole extracts of *F.hepatica*, liver tissue and purified GSTs were run on a vertical thin layer gel (final gel was12.5%). The gel was stained with 0.02% Coomassie Blue R-250 (mix 1 part filtered stock solution to 9 parts methanol: acetic acid: distilled water) (7). Ten micrograms of each protein was added to the gel. As a standard solution, Blue Pre-stained molecular weight marker (Novex) was used.

GSTs inhibition assay

GST inhibition by hexachlorophene as an inhibitor and 1-chloro-2,4-dinitrobenzene as a secondary substrate, was detected in a UV spectrophotometer at 340nm for GSTs assay of *F. hepatica* and liver eluted solutions (8).

RESULTS

Purified polypeptides eluted (Fractions1, 2) from the gel by addition of Tris-buffer showed GST activity. These eluted solutions were preserved for SDS-PAGE analysis. Polypeptides (Fractions 3,4,5) eluted from the gel by Tris-buffer showed lower activity and were used for comparative inhibitor enzyme assay. SDS-PAGE of homogenized and purified GSTs revealed a similar molecular weight. As shown in figure 1, the purified GSTs from *F. hepatica* and liver tissue have a molecular weight 26kDa.

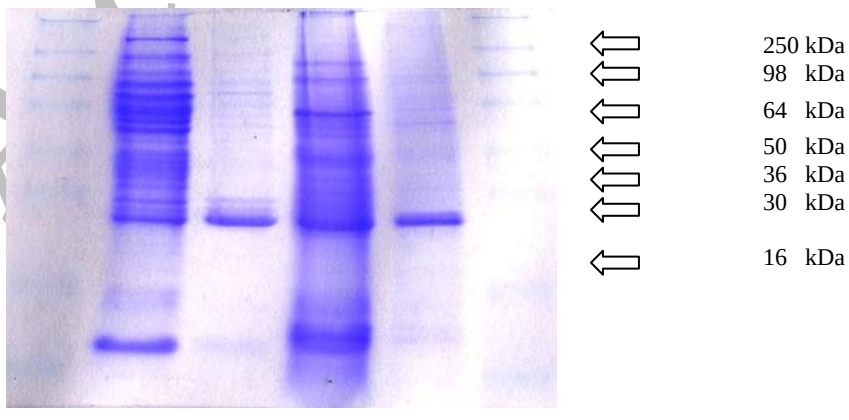
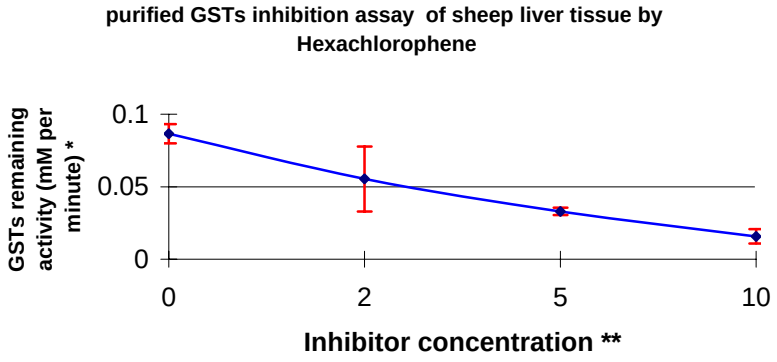
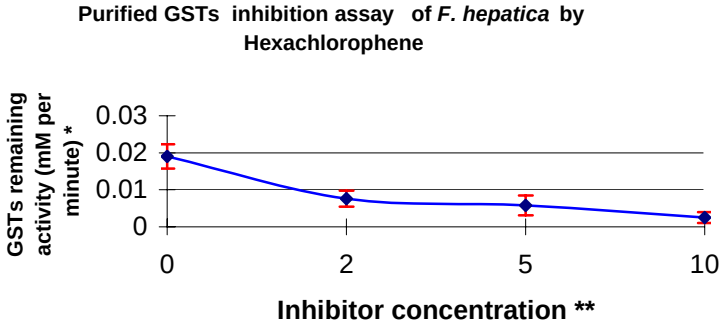


Fig. 1. SDS-Page of whole and purified extracts of GSTs from sheep liver tissue and *Fasciola hepatica.* Lane 1,marker sample; Lane 2,whole extract of *F.hepatica*; Lane 3, purified fraction of *F.hepatica* ; Lane 4, whole extract of sheep liver tissue; Lane 5, purified fraction of sheep liver tissue; Lane 6, marker sample.



IC50% value = 1µ M hexachlorophene

Fig. 2. Purified GSTs inhibition assay of liver tissue by hexachlorophene. * GSTs activity, with 1-chloro-2, 4-dinitrobenzene, was based on mM per minute and standard deviation of three assay values in each case. **IC50% value was based on micromole and three assays in each case.



IC50% value = 0.25 µM hexachlorophene

Fig. 3. Purified GSTs inhibition assay of *Fasciola hepatica* by hexachlorophene.* GSTs activity, with 1-chloro-2, 4-dinitrobenzene, was based on mM per minute and standard deviation of three assay values in each case. **IC50% value was based on micromole and three assays in each case.

The inhibitor concentration for the remaining 50% activity of liver GSTs was calculated graphically by Excel software and equalled 1µM hexachlorophene (Fig.2).

The inhibitor concentration for the remaining 50% activity of *F. hepatica* GSTs was calculated graphically by Excel software and equalled 0.25µM hexachlorophene (Fig.3).

Concentrations of the solubilized protein in homogenized whole extracts and purified eluted solutions were determined as 3.25 µg/ml and 3.25 µg/ml in sheep liver tissue and 6 µg/ml and 5 µg/ml in *F. hepatica*, respectively.

DISCUSSION

Glutathione affinity chromatography successfully isolated GST proteins from *F. hepatica* and sheep liver tissue. SDS-PAGE confirmed that *F. hepatica* GSTs migrate like liver tissue GSTs, as a band 26 kDa.

The hexachlorophene showed inhibitory activity against *F. hepatica* and sheep 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 (4 fold) as same as

whole extract tissue inhibition assay (8). This general inhibition of helminth and liver tissue GSTs in the micro molar range (as judged by IC50% value) by antihelmintics may help to explain the mode of action of these chemotherapeutic agents and sensitivity variation between two enzymes. The results suggest that GSTs of *F. hepatica* may be more sensitive than host liver tissue to hexachlorophene. A series of β-carbonyl substituted glutathione conjugates have been evaluated as inhibitors of *O. Volvulus* GST2 (9). Recent studies have shown *Piliostigma thonningii, Ocimum gratissium, Nauclea latifolia* (medicinal plants) containe heat-stable inhibitory activities against recombinant *Ascaris* and *Oncocerca* GSTs in vitro (10). However the binding of antihelmintics by a helminth GSTs may contribute to a passive detoxification mechanism (3).

In summary, although GSTs of *F. hepatica* and liver tissue show equivalent molecular weights, the remaining enzymatic activity has been shown to be different against hexachlorophene. As a model, the different effects of hexachlorophene on GSTs of *F.hepatica* and liver tissue could be concerned in the treatment of fascioliasis. Triclabendazole has recently been used as a drug of choice for human fascioliasis in Iran. Based on the results of this research, and for understanding the mechanism of triclabendazole's effect, GST inhibition assay of this drug is the next step of our research.

Acknowledgements

We would like to thank Prof. J. Barrett for the research facilities and Dr. J. R Jefferies, A. M. Campbell and A. J. Van Rossum for their help.

REFERENCES

1. Barrett J. Helminth detoxification mechanisms. J Helminthol. 1997; 71(2): 85-89.

2. Howell MJ, Board PG, Boray JC. Glutathione S-transferases in Fasciola hepatica. J Parasitol. 1988; 74(4): 715-718.

3. Brophy PM, Crowley P, Barrett J. Detoxification reactions of Fasciola hepatica cytosolic glutathione transferases. Mol Biochem Parasitol. 1990; 39(2): 155-162.

4. Brophy PM, Patterson LH, Brown A, Pritchard DI. Glutathione S-transferase (GST) expression in human hookworm Necator americanus: potential roles for excretory-secretory forms of GST. Acta Trop. 1995; 59(3):259-263.

5. Brophy P.M, Barrett J. Glutathione transferase in helminths. Parasitology. 1990; 100: 345-349.

6. Hillyer GV, Soler de Galanes M, Battisti G. Fasciola hepatica: host responders and nonresponders to parasite glutathione S-transferase. Exp Parasitol. 1992; 75(2): 176-186.

7. Salvatore L, Wijffels G, Sexton JL, Panaccio M, Mailer S, McCauley I, Spithill TW. Biochemical analysis of recombinant glutathione S-transferase of Fasciola hepatica. Mol Biochem Parasitol. 1995; 69(2): 281-288.

8. Farahnak A, Barrett J. Comparative glutathione S-Transferase (GSTs) inhibition assay in the whole extract of Fasciola hepatica and sheep liver tissue by hexachlorophene. Iranian J Publ Health. 2001; 30: 125-128.

9. Brophy PM, Campbell AM, van Eldik AJ, Teesdale-Spittle PH, Liebau E, Wang MF. Beta-carbonyl substituted glutathione conjugates as inhibitors of O. volvulus GST2. Bioorg Med Chem Lett. 2000; 10(9): 979-981.

10. Fakae BB, Campbell AM, Barrett J, Scott IM, Teesdale-Spittle PH, Liebau E, Brophy PM. Inhibition of gluthatione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother Res. 2000; 14(8): 630-634.