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REVIEW ARTICLE

Metabolic profiling of *Echinostoma caproni* and *Schistosoma mansoni* in their definitive and intermediate hosts

Ciara O'Sullivan, Bernard Fried* and Joseph Sherma

Department of Chemistry, *Department of Biology, Lafayette College, Easton, PA

Abstract

This review examines metabolic profiling of *Schistosoma mansoni* and *Echinostoma caproni* in their definitive and intermediate hosts. The earlier coverage of the literature on metabolic profiling was reviewed by Wang *et al*. 2010, *Advances in Parasitology*, 73, 373–404 and covered mainly studies using proton nuclear magnetic resonance spectroscopy. The methods focused upon in our review are mainly chromatographic. In the studies reviewed, various metabolites were analyzed in hosts infected with either *E. caproni* or *S. mansoni* and compared to the uninfected controls.

Keywords

Metabolic profiling, trematodes, *Schistosoma mansoni*, *Echinostoma caproni*, chromatography

Introduction

Studies of trematodes are important because millions of humans worldwide suffer from various diseases (trematodiases) associated with different species. These trematodes include blood flukes (*Schistosoma*), intestinal flukes (*Echinostoma*), and liver flukes (*Fasciola*, *Clonorchis*, and *Opisthorchis*) (Cox 1993). It is estimated that 40 to 50 million humans worldwide are infected with various food-borne trematodes, which include species of *Echinostoma* and *Fasciola* (Fried *et al*. 2004). *Schistosoma* infections cause schistosomiasis in humans, and it is estimated that schistosomes infect 200 million people globally (Chitsulo *et al*. 2000). Current methods of detecting and diagnosing trematodes mainly involve examination of the host feces for the presence of eggs (Fried *et al*. 2004). This is a time consuming and laborious process, and other methods of detection would be helpful to the biomedical community and to those who suffer from trematodiases. To help solve this problem, research on metabolic profiling has been undertaken to better understand how the presence of these trematodes affect host metabolism. Metabolic profiling determines the presence of certain metabolic reactions to specific outside stimuli (Wang *et al*. 2010, Holmes 2010). Studies analyzing such metabolic responses have been extensive and vital to the characterization of particular pathologies associated with metabolic and infectious diseases. The most recent review of metabolic profiling studies of trematode infections in humans is that of Wang *et al*. (2010). That review comprises the salient literature mainly based on nuclear magnetic resonance (NMR) spectroscopy methods to profile metabolic markers of major trematode infections. They compiled important information on the metabolic fingerprints of *S. mansoni*, *S. japonicum*, *E. caproni*, and *F. hepatica*, the major species of trematodes for which metabolic profiling studies are available.

There have been several reviews discussing food-borne trematodiasis, mainly on diseases associated with the following trematodes: *Echinostoma*, *Clonorchis*, *Fasciola*, *Opisthorchis*, and *Paragonimus*. Infection with these trematodes is usually caused from the consumption of metacercarial cyst contaminated freshwater aquatic animals including fish, frogs, snails, snakes, and tadpoles. The Fried *et al.* (2004), Fried and Abruzzi (2010) reviews include descriptions of these important trematodes, along with methods of diagnosis and treatment; current statistics on worldwide prevalence of these diseases are also included in these reviews.

*Corresponding author: friedb@lafayette.edu

In our review, we cover salient studies done after the review of Wang *et al*. (2010) and some studies not included in that review. The methods used in most of the papers we have included are mainly thin layer chromatography (TLC) or high performance TLC (HPTLC). The difference between these two methods is the type of plate used. In HPTLC, the sorbent particles are smaller and more uniform in size (mean particle size 5 μm), which results in better and faster separations and more sensitive detection of the studied analytes. All of the TLC/HPTLC studies reviewed involved use of a densitometer (CAMAG TLC Scanner 2 or 3 with Cats-3 or winCATS software, respectively) to scan standard and sample zones in the reflectance mode and automatically quantify the sample zones based on their scan areas compared to the areas of the standards in a calibration curve.

We only consider herein work on *S. mansoni* and *E. caproni*, since these two trematodes are the most widely studied for information on metabolic profiling. Within each section of our review, papers that have been covered first are studies of the infection in definitive hosts, followed by papers on the metabolic profiling in the intermediate host.

Schistosoma Studies

Trematodes of the genus *Schistosoma* have been extensively studied because they cause schistosomiasis, a tropical disease infecting millions of people globally. Adult schistosomes are white to gray, dioecious worms, 7–20 mm long with two terminal suckers (Gryseels *et al*. 2006) and live in the mesenteric and hepatic portal blood vessels of their vertebrate hosts. These blood flukes are endemic to 74 countries and territories primarily in Africa, the Arabian Peninsula, South America, and Asia (Chitsulo *et al*. 2000). The main species infecting humans are *S. mansoni*, *S. japonicum*, and *S. haematobium* (Gryseels *et al*. 2006). Because the metabolic profiling literature is mainly on *S. mansoni*, this is the only schistosome we cover here.

Muller *et al*. (2001) examined the effects of *S. mansoni* infection on the neutral lipid content of the liver, ileum, and serum of mice. Host samples were examined at days 14, 45, and 75 post infection. After sample preparation by the Folch method [extraction of lipids with chloroform-methanol (2:1) followed by purification of the extracts by washing with 0.88% aqueous KCl], lipids were separated by HPTLC on Whatman LHPKDF laned preadsorbent silica gel plates with the Mangold (1969) mobile phase (petroleum ether-diethyl ether-glacial acetic acid, 80:20:1) and detected as dark blue zones on the yellow layer background using 5% ethanolic phosphomolybdic acid (PMA) spray reagent. The most significant finding was that triacylglycerol content in the liver and ileum decreased as the infection progressed, and at the same time cholesterol esters in the liver decreased.

O'Sullivan *et al*. (2012) examined the effects of *S. mansoni* infection on the neutral and polar lipids of the liver, spleen, and small intestine of mice. The samples were analyzed after the mice were necropsied 8 weeks post-infection. Neutral lipids isolated by Folch extraction were separated on preadsorbent, laned Analtech HPTLC-HLF silica gel plates with the Mangold mobile phase and were detected using PMA reagent agent. Polar lipids were separated with the Wagner *et al*. (1961) mobile phase chloroform-methanol-deionized (DI) water (65:25:4) and detected using 10% cupric sulfate in 8% phosphoric acid spray reagent. These lipids appeared as brown-black spots on a white layer background. No significant differences were found in the neutral lipids of the infected mice compared to the uninfected controls. There was a significantly lower amount of the polar lipid phosphatidylcholine in the liver and small intestine of infected mice compared to uninfected controls.

Muller *et al*. (2000) studied the effects of *S. mansoni* infection on the neutral lipids of the digestive gland-gonad complex (DGG) of *B. glabrata*. The lipids were separated on Whatman LHPKDF silica gel HPTLC plates with the Mangold mobile phase and detected with PMA reagent. There was a significant increase in the free sterol and triacylglycerol fractions in the DGG at six and eight weeks post-infection of infected snails compared to the uninfected controls.

Pachuski *et al*. (2002) studied the effects of *S. mansoni* infection on the amino acid content of the DGG of *B. glabrata* snails. Amino acids were extracted with ethanol-DI water (70:30) and separated on Whatman LHPKDF silica gel HPTLC plates, strong acid cation-exchange sheets (POLYGRAM IONEX-25 SA-Na; Macherey-Nagel), reversed phase plates with preadsorbant zone (Whatman LKC18F), and HPTLC cellulose F plates (EMD Millipore, a division of Merck KgaA). Three different mobile phases were used: pH 3.3 citrate buffer for the ion exchange sheet, n-propanol-0.5M NaCl (4:6) for the reversed phase layer, and n-butanol-acetic acid-water (3:1:1) for both silica and cellulose gel layers. Detection of the amino acids was done with ninhydrin reagent to produce purple zones for all amino acids except proline, which showed as a yellow zone. There was a significant decrease in the amount of lysine in the infected snails compared to the uninfected controls.

In one non-chromatographic study not included in the Wang *et al*. (2010) review, Ong *et al*. (2004) used inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to study the effects of *S. mansoni* infection on the inorganic elements in *B. glabrata* snails. A Thermo Jarrell Ash simultaneous-reading ICP-AES instrument was used to measure the concentrations of 28 inorganic elements in both infected and uninfected snail bodies. Elevated concentrations of calcium, cadmium, manganese, and sodium in the bodies of infected snails were found compared to the uninfected controls.

Jarusiewicz *et al*. (2006) studied the glucose and maltose content in the DGG of estivated *B. glabrata* snails infected with *S. mansoni* compared to estivated, uninfected controls. The sugars were extracted using 70% aqueous ethanol, and TLC was done on Whatman LK5DF laned preadsorbent silica gel plates developed with ethyl acetate-glacial acetic acid-methanol-DI water (60:15:15:10) mobile phase. The carbo-

hydrates were detected using the α-naphthol-sulfuric acid reagent described in Cline *et al*. (1999). Spots appeared as dark purple zones on a yellow layer background. Both infected and uninfected estivated snails showed significantly decreased maltose and glucose levels compared to the non-estivated infected and uninfected controls.

Massa *et al*. (2007) examined the effects of *S. mansoni* infection on the carboxylic acids of *B. glabrata* using ion exclusion high performance liquid chromatography (HPLC). They analyzed both the snail DGG and hemolymph. HPLC was carried out using an Aglient 1100 series system with an autosampler, a Bio-Rad Laboratories Aminex ion exclusion HPX-87H column, and an ultraviolet diode array detector. Sulfuric acid (5.0 mM) was the mobile phase with a flow rate of 0.6 mL/min. Retention times of the standard and sample acids were matched to identify each acid. They found that there was a significant reduction in all of the carboxylic acids in the DGG of infected snails compared to uninfected controls. This study suggests that carboxylic acids studied are useful metabolic indicators of infection in the snails.

Echinostoma Studies

Trematodes in the genus *Echinostoma* have been extensively studied because they have economic importance in causing food-borne trematodiases. These trematodes are intestinal flukes, 3–10 mm long and 1–3 mm wide with a large ventral sucker (Fried *et al*. 2004). They are endemic in many Southeastern Asian countries and infect numerous humans who inadvertently ingest the metacercarial cysts of echinostomes in freshwater and brackish water molluscs, fish, crustaceans and amphibians (Fried *et al*. 2004). The most widely used laboratory model is *E. caproni* because it has a relatively short time to patency (10–14 days) in mice and is an easy species to maintain in the laboratory since it cycles between *B. glabrata* and laboratory mice or hamsters (Fried and Huffman 1996). There have been numerous chromatographic studies on the changes in various metabolites associated with *E. caproni* infection in both mice and snails.

Bandstra *et al*. (2007) used HPTLC to determine the neutral lipid content of mice feces 1–5 weeks postinfection. Samples and standards were chromatographed on either Whatman LHPKDF silica gel plates or EMD Millipore silica gel 60 CF_{254} plates developed with the Mangold mobile phase. The neutral lipids were detected using PMA reagent. There was a significant decrease in the triacyglycerol fraction compared to uninfected controls at 3 weeks post-infection. A significant increase in the free sterol fraction at 2 weeks postinfection compared to uninfected controls also occurred.

Murray *et al*. (2007) examined the phospholipid and sphingolipid content in the feces of mice infected with *E. caproni* using HPTLC. Samples and standards were chromatographed on EMD Millipore silica gel 60 CF_{254} plates developed with chloroform-methanol-DI water (65:225:4), and polar lipids were detected using a 10% cupric sulfate in 8% phosphoric acid reagent. The fecal polar lipids were not useful to analyze as markers of an *E. caproni* infection since no significant differences were detected between the infected and uninfected samples.

Massa *et al*. (2008) extended the study of Bandstra *et al*. (2007) and used the same HPTLC procedures to examine the neutral lipid content of mice feces at 1–7 weeks postinfection. Analysis was done weekly on the fecal samples and consisted of an examination of the free fatty acids, free sterols, triacylglycerols, methyl esters, and steryl esters. At 7 weeks postinfection, the triacylglycerol content was significantly lower in infected mice than in the uninfected controls.

Vasta *et al*. (2008) used HPTLC to examine the neutral lipid content of the mice urine infected with *E. caproni*. Folch extracted samples and standards were chromatographed on EMD Millipore silica gel 60 CF_{254} plates developed with the Mangold mobile phase; bands were detected using PMA reagent as described in Bandstra *et al*. (2007). To confirm or reject the presence of methyl and steryl esters, the Smith *et al*. (1995) mobile phase consisting of hexanes-petroleum ether-diethyl ether-glacial acetic acid (50:20:5:1), was used on the samples. Vasta *et al*. (2008) found that methyl esters were significantly increased at 6 and 7 weeks postinfection compared to the uninfected controls.

Massa *et al*. (2008) analyzed the polar lipid content of human urine, uninfected mouse urine, and urine from mice infected with *E. caproni*. The purpose of this study was to determine if the mouse model showed comparable polar lipid profiles to human urine. Lipids were separated on Whatman LHPKDF and EMD Millipore silica gel 60 CF_{254} HPTLC plates with the Wagner mobile phase and zone detection with 10% cupric sulfate in 8% phosphoric acid reagent. No significant differences in the polar lipids between infected and noninfected mice were found. The polar lipid profiles of human and mouse urine were different.

Vasta *et al*. (2009) used HPTLC to analyze the amino acids in the urine of mice infected with *E. caproni*. Ethanol-DI water (70:30) sample extracts and standards were chromatographed on both EMD Millipore HPTLC silica gel and cellulose layers. The silica gel layer with a preadsorbent zone was No. 13728-6 and the cellulose layer was No. 15036-6. Plates were developed with one of two mobile phases: mobile phase A consisted of 2-butanol-pyridine-glacial acetic acid-DI water (39:34:10:26) and mobile phase B consisted of 2-butanol-pyridine-25% ammonia-DI water (39:34:10:26). Zones were detected using ninhydrin reagent and they appeared in various colors on a white to pink background, which aided zone identification. Vasta *et al*. (2009) found that taurine, alanine, threonine, and lysine were present in mouse urine and that alanine and taurine were present in significantly different amounts in infected and uninfected mice. Some of these findings correlated with the spectroscopic findings of Saric *et al*. (2008) who suggested that taurine levels in urine may serve as a biological marker for mice infected with *E. caproni* using spectroscopic methods.

Counihan *et al*. (2010) examined the neutral and polar lipids in intestinal and non-intestinal organs of mice infected with *E. caproni*. The Folch extracted samples and standards were chromatographed on Analtech HPTLC-HLF silica gel plates with the Mangold mobile phase, and detection of zones was carried out with PMA reagent. Polar lipids were developed with the Wagner *et al*. (1961) mobile phase and detected with 10% cupric sulfate in 8% phosphoric acid reagent. The authors found that there were no significant differences in the neutral lipids of the infected mice compared to the uninfected controls, but the polar lipid phosphatidylethanolamine in the anterior portion of the small intestine was found in significantly higher amounts in the infected mice compared to the uninfected controls.

Bandstra *et al*. (2006) determined neutral lipids and phospholipids in *B. glabrata* patently infected with *E. caproni*. The DGG, hemolymph, and shells were analyzed. Folch extracted samples and standards were chromatographed on Whatman LHPKDF-HPTLC silica gel plates with the Mangold mobile phase and zones were detected using PMA reagent. To separate the steryl esters, the Smith *et al*. (1995) mobile phase was used. Detection was the same as for the other neutral lipids. Phospholipids were developed with the Wagner *et al*. (1961) mobile phase and detected using 10% cupric sulfate in 8% phosphoric acid reagent. Infected snails had significantly lower amounts of triacylglycerols in the DGG compared to uninfected controls. There were no significant differences in the shells or plasma samples in any of the lipid classes.

Concluding Remarks

Extensive work has been carried out using various analytical methods on metabolic profiling of *E. caproni* and *S. mansoni*. Examining the effects of trematode infections on the different metabolites in both the intermediate and definitive hosts is an important area of work as researchers continue to try and discover simpler and better methods to identify trematode infections in humans and animal.

References

Bandstra S.R., Murray K.E., Fried B., Sherma J. 2007. High performance thin layer chromatographic analysis of neutral lipids in the feces of BALB/c mice infected with *Echinostoma caproni*. *Journal of Liquid Chromatography and Related Technologies*, 30, 1437–1445. DOI: 10.1080/10826070701277034.

Bandstra S.R., Fried B., Sherma J. 2006. High performance thin layer chromatographic analysis of neutral lipids and phospholipids in *Biomphalaria glabrata* patently infected with *Echinostoma caproni*. *Parasitology Research*, 99, 414–418. DOI: 10.1007/s00436-006-0180-5.

Chitsulo L., Engels D., Montresor A., Savioli L. 2000. The global status of schistosomiasis and its control. *Acta Tropica*, 77, 41–51. DOI: 10.1016/S0001-706X(00)00122-4.

Cline D.J., Fried B., Sherma J. 1999. High performance thin layer chromatography determination of carbohydrates in the hemolymph and digestive gland of *Lymnaea elodes* (Gastropoda: Lymnaeidae). *Veliger*, 42, 185–188.

Counihan J., Fried B., Sherma J. 2010. Effects of *Echinostoma caproni* infection on the neutral and polar lipids of intestinal and non-intestinal organs in the BALB/c mouse as determined by high performance thin layer chromatography. *Parasitology Research*, 107, 947–953. DOI: 10.1007/s00436-010-1959-y.

Cox F.E.G. 1993. Modern Parasitology: A Textbook for Parasitology. Blackwell Science, Ltd, Oxford, UK.

Fried B., Abruzzi A. 2010. Food borne trematode infections of humans in the United States of America. *Parasitology Research*, 106, 1263–1280. DOI: 10.1007/s00436-010-1807-0.

Fried B., Graczyk T.K., Tamang L. 2004. Food-borne intestinal trematodiasis in humans. *Parasitology Research*, 93, 159–170. DOI: 10.1007/s00436-004-1091-y.

Fried B., Huffman J.E. 1996. The biology of the intestinal trematode *Echinostoma caproni*. *Advances Parasitology*, 38, 311–368. DOI: 10.1016/S0065-308X(08)60037-8.

Gryseels B., Polman K., Clerinx J., Kestens L. 2006. Human schistosomiasis. *Lancet*, 368, 1106–1118. DOI: 10.1016/S0140-6736(06)69440-3.

Holmes E. 2010. The evolution of metabolic profiling in parasitology. *Parasitology*, 137, 1437–1449. DOI: 10.1017/S0031182010000260.

Jarusiewicz J., Sherma J., Fried B. 2006. Thin layer chromatographic analysis of glucose and maltose in estivated *Biomphalaria glabrata* snails and those infected with *Schistosoma mansoni*. *Comparative Biochemistry and Physiology*, 145B, 3–4, 346–349. DOI: 10.1016/j.cbpb.2006.08.008.

Mangold H.K. 1969. Thin-layer chromatography, 2nd edn. Springer, New York.

Massa D.R., Chejlava M.J., Fried B., Sherma J. 2007. High performance column liquid chromatographic analysis of selected carboxylic acids in *Biomphalaria glabrata* patently infected with *Schistosoma mansoni*. *Parasitology Research*, 101, 925–928. DOI: 10.1007/s00436-007-0563-2.

Massa D.R., Vasta J.D., Fried B., Sherma J. 2008. High performance thin-layer chromatographic analysis of the polar lipid content of human urine and urine from BALB/c mice experimentally infected with *Echinostoma caproni*. *Journal of Planar Chromatography – Modern TLC*, 21, 337–341. DOI: 10.1556/JPC.21.2008.5.4.

Muller E., Brunet L.R., Fried B., Sherma J. 2001. Effects on the neutral lipids contents of the liver, ileum and serum during experimental schistosomiasis. *International Journal for Parasitology*, 31, 285–287. DOI: 10.1016/S0020-7519(00)00171-5.

Muller E., Fried B., Sherma J. 2000. HPTLC analysis of neutral lipids in *Biomphalaria glabrata* snails infected with *Schistosoma mansoni*. *Journal of Planar Chromatography – Modern TLC*, 13, 228–230.

Murray K.E., Fried B., Sherma J. 2007. Determination of the phospholipid and sphingolipid content in the feces of uninfected BALB/c mice and those infected with *Echinostoma caproni* by high performance thin layer chromatography with densitometry. *Acta Chromatographica*, 18, 190–198.

Ong J.H.L., Chejlava M., Fried B., Koehnlein K.M., Bosavage G.L., Sherma J. 2004. Effects of *Schistosoma mansoni* infection on the inorganic elements in the snail *Biomphalaria glabrata*. *Journal of Helminthology*, 78, 343–346. DOI: 10.1079/JOH2004244.

O'Sullivan C., Popovic N., Fried B., Sherma J. 2012. Effect of *Schistosoma mansoni* on the neutral and polar lipids in the liver, spleen and small intestine of mice as determined by high performance thin layer chromatography. *Journal of Planar Chro-*

matography – Modern TLC, 25, 259–261. DOI: 10.1556/JPC.25.2012.3.12.

Pachuski J., Fried B., Sherma J. 2002. HPTLC analysis of amino acids in *Biomphalaria glabrata* infected with *Schistosoma mansoni*. *Journal of Liquid Chromatography and Related Technologies*, 25, 2345–2349. DOI: 10.1081/JPC-120014008.

Saric J., Li J.V., Wang Y.L., Keiser J., Bundy J.G., Holmes E., Utzinger J. 2008. Metabolic profiling of an *Echinostoma caproni* infection in the mouse for biomarker discovery. *Plos Neglected Tropical Diseases*, 2, No. e 254. DOI: 10.1371/journal.pntd.0000254.

Smith M.C., Webster C.L., Sherma J., Fried B. 1995. Determination of neutral lipids in regular and lowfat eggs by high-performance TLC with densitometry. *Journal of Liquid Chromatography and Related Technologies*, 18, 527–535. DOI: 10.108026079508009254.

Vasta J., Fried B., Sherma J. 2008. High performance thin layer chromatographic analysis of neutral lipids in the urine of BALB/c mice infected with *Echinostoma caproni*. *Parasitology Research*, 192, 625–629. DOI: 10.1007/s00436-007-0798-y.

Vasta J., Fried B., Sherma J. 2009. Determination and quantification of amino acids in the urine of BALB/c mice infected with *Echinostoma caproni* by high performance thin layer chromatography-densitometry. *Journal of Liquid Chromatography and Related Technologies*, 32, 1210–1222. DOI: 10.1080/10826070902854573.

Wagner H., Horhammer L., Wolff P. 1961. Thin-layer chromatography of phosphatides and glycolipids. *Biochemische Zeitschrift*, 334, 175.

Wang Y., Li J.V., Saric J., Keiser J., Wu J., Utzinger J., Holmes E. 2010 Advances in metabolic profiling of experimental nematode and trematode infections. *Advances in Parasitology*, 73, 373–404. DOI: 10.1016/S0065-308X(10)73012-8.

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