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Changes in the expression of cytochrome P450 isozymes and related carcinogen metabolizing enzyme activities in *Schistosoma mansoni*-infected mice

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Abstract

Mixed-function oxidase enzymes metabolize most xenobiotic agents. Western blotting was used to investigate the effect of *Schistosoma mansoni* infection on the expression of various cytochrome P450 (CYP) isozymes and specific enzyme assays to study related metabolic functions in mouse liver microsomes. Male BK-TO mice were infected with 200 cercariae per mouse and their livers were assayed at 6, 15, 30 and 45 days post-infection (p.i.) and compared with appropriately matched controls. The expression of each of the CYP isozymes (1A1, 2B1/2, 2C6, and 4A) was either unaffected or transiently increased up to 30 days post-infection. By 45 days, a significant loss of signal was observed, particularly for CYP 1A1 and 2B1/2 where no signal could be detected. Evidence supporting these findings was obtained from enzyme assays specific for particular CYP isozymes. The activity of ethoxyresorufin *O*-deethylase (CYP 1A1) was reduced by 97% and that of pentoxyresorufin *O*-dependentylase (CYP 2B1/2) by 96% at 45 days p.i. Similarly, the activity of ethoxycoumarin hydroxylase was progressively reduced over the period under study. It is believed that *N*-nitrosamines are activated principally by *N*-nitrosodimethylamine *N*-demethylase I which was significantly increased at both 30 and 45 days p.i. To further investigate metabolic competency following *S. mansoni* infection, the *in vitro* binding of benzo(*a*)pyrene metabolites to DNA was measured, using isolated liver microsomes to activate benzo(*a*)pyrene. Benzo(*a*)-pyrene-DNA adduct formation was markedly increased at 6, 15 and 30 days with a maximum at 15 days, but decreased at 45 days p.i. It was concluded that *S. mansoni* infection changes the expression of different CYP isozymes and also the activity of phase I drug-metabolizing enzymes at different periods of infection and may thus change the liver's capacity to activate or detoxify many endogenous and exogenous compounds. Such alterations may also change the therapeutic actions of drugs that are primarily metabolized by the P450 system, when administered to patients with schistosomiasis.

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Introduction

Schistosomiasis is a widespread endemic parasitic disease affecting agricultural communities in 75 subtropical and tropical countries (WHO, 1985) with estimates of more than 200 million people infected and at least 500 million at the risk of infection (WHO, 1986). In Egypt, schistosomiasis heads the list of endemic parasitic diseases with regard to prevalence and intensity of infection; 60% of the Egyptian population is exposed to the risk of the disease and many health problems are associated with schistosomiasis, including weakness, diarrhoea, loss of weight, difficulty in urination, hepatosplenomegaly and carcinoma of the intestine, bladder and liver (Hashem & Boutros, 1961; Cheever, 1978; Delmas *et al.*, 1986; Al-Shukri *et al.*, 1987).

The hepatic cytochrome P450s (CYP) are a multigene family of enzymes that play a critical role in the metabolism of many drugs and xenobiotics with each CYP isozyme responding differently to exogenous chemicals in terms of its induction and inhibition (Sheweita, 2000). Thus, CYP 1A1 is particularly active towards polycyclic aromatic hydrocarbons (PAHs), activating them into reactive intermediates that covalently bind to DNA, a key event in the initiation of carcinogenesis (Jerina *et al.*, 1979; Manchester *et al.*, 1992). Likewise, CYP 1A2 activates a variety of bladder carcinogens, such as aromatic amines (Hammons *et al.*, 1985; Butler *et al.*, 1989). Polycyclic aromatic hydrocarbons are of special health concern because they are present both in the environment and in certain occupational situations and some are believed to cause cancer in humans (Harris *et al.*, 1985; Everson *et al.*, 1986; Savela & Hemminki, 1991). An important and extensively studied member of this class of compound is benzo(*a*)pyrene (B(*a*)P), which has been shown to cause cytotoxic, mutagenic and carcinogenic effects in tissues from various animal species (Conney, 1982; Ashurst & Cohen, 1981; Gonzalez & Gelboin, 1994; Lake *et al.*, 1996). It has been reported that the carcinogenic potency of B(*a*)P and the extent of binding of its ultimate metabolites to DNA and protein is correlated with the induction of aryl hydrocarbon hydroxylase (AHH) and cytochrome P450 (Conney, 1982; Gooderham & Mannering, 1985).

N-nitrosamines are carcinogenic compounds that occur widely in the environment and can be formed endogenously from the interaction of ingested nitrate or nitrite with secondary amines (Lijinsky *et al.*, 1972). Their role as causative agents in the carcinogenesis of some human neoplastic diseases has been extensively reviewed (Preussmann, 1984; Bartsch & Montesano, 1984; Hill *et al.*, 1988). *N*-nitrosamines also require metabolic activation in order to exert their cytotoxic and carcinogenic effects and there are at least two enzyme species responsible for their *N*-demethylation, namely *N*-nitrosodimethylamine-*N*-demethylases I and II (NDMA dI and dII) that can operate at 4 and 100 mM of NDMA concentration (Arcos *et al.*, 1977; Mostafa & Sheweita, 1992). Cytochrome P450 2E1 is the main CYP isozyme involved in the *N*-demethylation of NDMA and also in the metabolism of a wide range of small organic molecules such as ethanol, benzene and carbon tetrachloride (Yoo *et al.*, 1988; Camus *et al.*, 1993). Following the *N*-demethylation of NDMA, a diazonium ion is produced leading ultimately to the formation of a carbonium ion, which is the metabolite that methylates DNA and other macromolecules (Umbenhauer *et al.*, 1985; Cooper *et al.*, 1991).

In mice, *Schistosoma mansoni* infection results in a transient increase in the activity of some drug-metabolizing enzymes during the early stages of infection, followed by an overall reduction at the later stages (El-Mouelhi *et al.*, 1987; Sheweita & Mostafa, 1995; Sheweita *et al.*, 1998). Moreover, marked decreases in total CYP content and glutathione S-transferase activity have also been observed in *S. mansoni*-infected human livers during the later stages of infection (Habib *et al.*, 1996; Sheweita *et al.*, 1997). This inhibition of CYP content at later stages of *S. mansoni* infection has resulted in the reduced binding of 2-acetylaminofluorene (2-AAF) and aflatoxin B_1 to mouse liver macromolecules such as DNA and protein (Hasler *et al.*, 1986; Siwela *et al.*, 1990).

Previous studies have demonstrated the influence of *S. mansoni* on the total content of CYP (Sheweita & Mostafa, 1995; Sheweita *et al.*, 1998). The present study shows the changes both the cellular levels of the individual CYP isozymes (1A1, 2C6, 2B1/2, and 4A) and their associated enzyme activities (ethoxycoumarin hydroxylase, ethoxyresorufin *O*-deethylase, and pentoxyresorufin *O*-depentylase) in *S. mansoni*-infected mouse liver microsomes.

Materials and methods

Chemicals

Benzo(*a*)pyrene was obtained from Koch-light laboratories, England and all other chemicals from Sigma (Poole, UK). Anti-cytochrome P450 1A1, 2B1/2 and 4A Western blotting kits were purchased from Amersham International, UK. Anti-cytochrome P450 2C6 was kindly provided by Dr Harry Gelboin, National Cancer Institute, Maryland, USA

Experimental infection of mice

Male BK-TO mice weighing 20–25 g were infected with *S. mansoni* cercariae by direct exposure to 200 cercariae per mouse as described by Smithers & Terry (1965). Counting of cercariae after infection was performed. The infected mice were killed at 6, 15, 30 and 45 days post-infection (p.i.) and assayed with the corresponding control group. The numbers of mice used at each time point were six infected and six controls.

Enzyme assays

Mice were killed by cervical dislocation, the liver dissected and homogenized in three volumes of 0.1 M potassium phosphate buffer, pH 7.4, and centrifuged at 12,000 *g* for 20 min at 4°C. The supernatant was centrifuged at 105,000 *g* for 1 h at 4°C to yield a microsomal pellet which was then resuspended in 0.1 M potassium phosphate buffer, pH 7.4 (Gelboin, 1980; Sheweita *et al.*, 1998). Protein concentration was measured by the method of Lowry *et al.* (1951), using BSA as standard.

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Microsomal NDMA-dI activity was determined according to the method of Venkatesan *et al.* (1968), with the modifications of Mostafa & Sheweita (1992). Substrate concentration was 4 mM NDMA, which represents the saturation level for NDMA-dI. The amount of formaldehyde formed was determined by the method of Nash (1953), with the modification of Mclean & Day (1974). The enzymatic activity of NDMA-dI was expressed as nmol of formaldehyde per mg protein per hour and corrected for inhibition caused at this concentration of NDMA by the inclusion of semicarbazide in the assay mixture to prevent loss of formaldehyde (Yoo *et al.*, 1988).

Ethoxycoumarin hydroxylase activity was assayed by the method of Greenle & Poland (1978). The intensity of 7-hydroxycoumarin fluorescence was measured at excitation and emission wavelengths of 338 and 458 nm, respectively. Ethoxyresorufin *O*-deethylase and pentoxyresorufin *O*-dependentylase activity were determined by the methods of Pohl & Fouts (1980) and Burke *et al.* (1985), respectively. Product concentration was interpolated from a standard curve for resorufin.

Western blotting

Ten to thirty micrograms of total microsomal protein was subjected to SDS-polyacrylamide gel electrophoresis. Proteins were electroblotted to Hybond-C membrane and specific CYP isozymes detected using the appropriate cytochrome P450 ECL Western blotting kit, following the manufacturer's instructions (Amersham International, UK). Band density was assessed using a UVP Imagestore 5000 system (Ultraviolet Products Ltd, UK).

Assay of benzo(a)pyrene-DNA adducts

The formation of benzo(*a*)pyrene-DNA adducts was carried out as described by Vahakangas *et al.* (1989) and Bjelogrlic *et al.* (1993). DNA-bound benzo(*a*)pyrene tetrols and triols were measured using a synchronous fluorescence spectrophotometer at 344 nm.

Statistical analysis

Each enzyme assay was performed twice and the mean and standard errors calculated. Data were compared by a Student's t-test and the level of significance for all experiments was set at $P < 0.05$.

Results

All CYP isozymes monitored showed some degree of reduced expression by 45 days p.i. with *S. mansoni* (fig. 1). This was most marked for CYP 1A1 and CYP 2B1/2 (fig. 1A and B, respectively) for both of which no polypeptide band could be detected. For CYP 2C6 and CYP 4A, band intensities were reduced by 57% and 32%, respectively, when compared with non-infected control lanes (fig. 1C and D).

To confirm the results of the Western blot analyses, a number of CYP specific enzyme assays were undertaken using the same liver microsome samples. To test for the presence of CYP 1A1, liver samples were assayed for ethoxyresorufin *O*-deethylase and ethoxycoumarin hydroxylase (table 1). For the former, a gradual decrease in specific activity was observed with increasing times p.i., while activity in control samples remained virtually unchanged (table 1). The virtual ablation of activity at 45 days p.i. correlates well with the Western blot result, where no band corresponding to the CYP 1A1 polypeptide was observed. Although not specific to the CYP 1A1 protein, ethoxycoumarin hydroxylase activity is also a good marker for this CYP isozyme. Again, activity was reduced in *S. mansoni*-infected samples, however, less so than for ethoxyresorufin *O*-deethylase, reflecting the multiplicity of proteins capable of catalysing this enzyme reaction (table 1).

Similarly, the activity of pentoxyresorufin *O*-dependentylase, associated with CYP 2B1/2, was determined over the time course of the present study. Although activity was initially significantly higher than that of controls (table 2), it was subsequently markedly reduced and almost ablated at 45 days p.i., again correlating well with the lack of signal by Western blotting. The activity of NDMA-dI was measured in *S. mansoni*-infected and control liver microsomes. This activity tends to increase by 15 days p.i. and reaches a maximum at 45 days p.i. (table 2).

As an alternative measure of CYP 1A1 activity, the *in vitro* formation of benzo(*a*) pyrene-DNA adducts was determined, using liver microsomal samples to activate B(*a*)P. In contrast with the results obtained for ethoxyresorufin *O*-deethylase activity, a transient increase in adduct formation, peaking at 15 days, was detected during the early stages of infection. However, liver

Table 1. The effect of *Schistosoma mansoni* infection on the activities of ethoxyresorufin *O*-deethylase and ethoxycoumarin *O*-deethylase in mouse liver microsomes.

	Ethoxyresorufin *O*-deethylase (nmole resourfin/mg protein/min)[a]		Ethoxycoumarin hydroxylase (n moles hydroxycoumarin/mg protein/min)[a]	
Days post-infection	Control	Infected	Control	Infected
6	11.6±0.2	10.6±1.9 (NS)[b]	0.988±0.014	0.74±0.093 (−25%, $P<0.05$)
15	11.4±1.0	7.5±0.3 (−34%, $P<0.005$)	0.962±0.016	0.766±0.11 (−20% $P<0.05$)
30	11.9±0.8	5.4±0.3 (−55%, $P<0.001$)	0.955±0.019	0.68±0.033 (−32%, $P<0.001$)
45	9.9±0.5	0.3±0.1 (−97%, $P<0.001$)	1.03±0.0065	0.624±0.007 (−40% $P<0.001$)

[a] Means ± SE of six mice.
[b] NS, not significant statistically.

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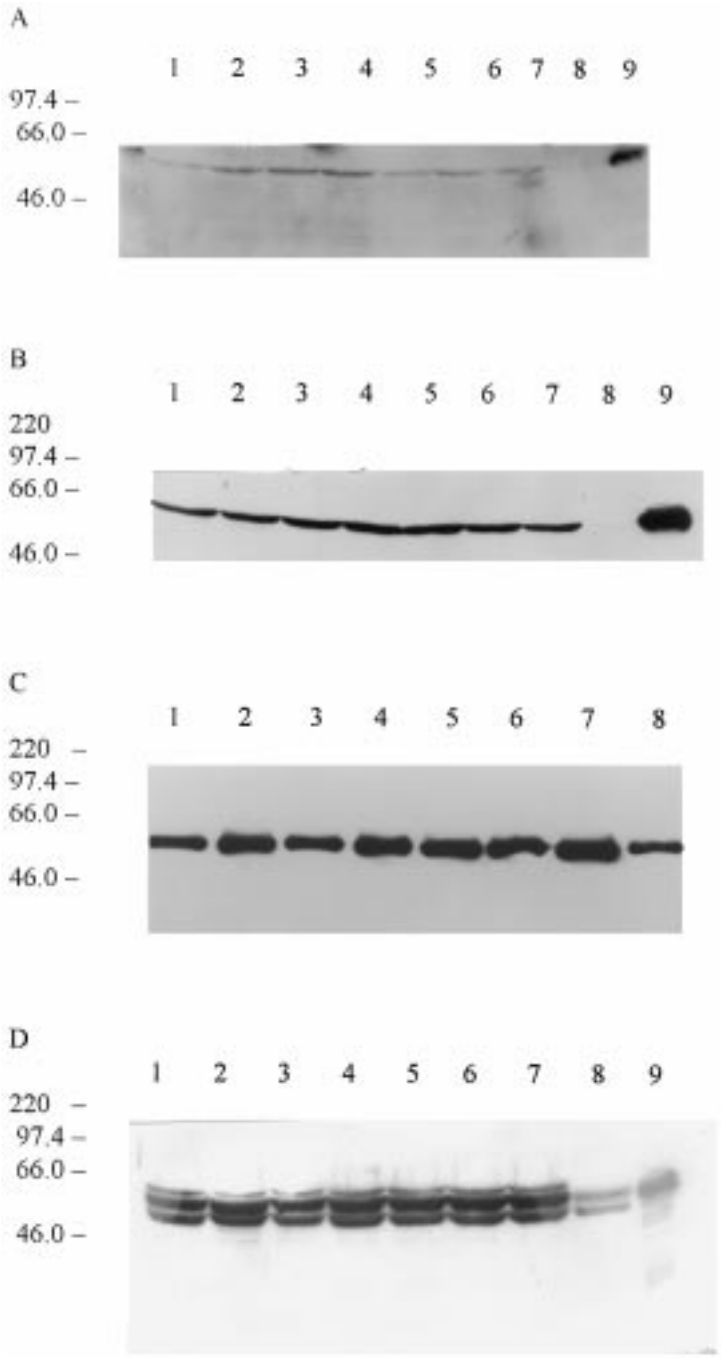

Fig. 1. Western blot analysis of the influence of *Schistosoma mansoni* infection on the expression of cytochrome P450 isozymes. Lanes 1, 3, 5 and 7 are microsomal protein of matched control groups at 6, 15, 30 and 45 days. Lanes 2, 4, 6 and 8 are microsomal proteins of infected mice at 6, 15, 30 and 45 days post-infection. A positive control sample was also run (lane 9). (A) CYP 1A1, (B) CYP 2B1/2, (C) CYP 2C6, (D) CYP 4A.

samples from *S. mansoni*-infected mice showed a much reduced capacity to activate B(*a*)P at 45 days p.i. (table 3). These differences might well reflect the balance of induction of enzymes of bioactivation and deactivation.

Discussion

Variations in the activity of CYP isozymes are well correlated with deleterious effects of chemical carcino-

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Table 2. The effect of *Schistosoma mansoni* infection on the activities of pentoxyresourfin *O*-depentylase and NDMA- *N*-demethylase I in mouse liver microsomes.

	Pentoxyresourfin *O*-depentylase (nmole resourfin/mg protein/min)[a]		NDMA-d I (nmole HCHO/mg protein/h)[a]	
Days post-infection	Control	Infected	Control	Infected
6	4.9±0.9	6.2±0.6 (+26%, NS)[b]	129±3.7	134±0.7 (NS)
15	4.6±0.8	7.0±0.5 (+52%, $P<0.05$)	134±1.0	148±3.6 (NS)
30	5.1±0.7	3.8±0.3 (−25%, $P<0.05$)	130±2.2	168±1.0 (+30%, $P<0.001$)
45	4.5±0.3	0.2±0.01 (−96%, $P<0.001$)	133±1.8	234±3.2 (+76%, $P<0.001$)

[a] Mean ± SE of six mice.
[b] NS, not significant statistically.

gens, such as *N*-nitrosamines, which are primarily metabolized in the liver by hepatic microsomal NDMA-dI (Yang *et al.*, 1990; Yoo *et al.*, 1990). In the present study changes in the cellular levels of specific cytochrome P450 isozymes following *S. mansoni*-infection in the mouse liver are presented for the first time. NDMA-dI activity was markedly induced in liver microsomes from *S. mansoni*-infected mice at relatively late stages of infection (30 and 45 days). At such times therefore, the deleterious effects of *N*-nitrosamines might be increased in the liver, the primary site of activation, or in other organs such as the bladder. Supporting this suggestion, it has been found that inhibition of NDMA-dI was effective in decreasing the tumourigenicity of *N*-nitrosamines in rodents (Wattenberg, 1987; Ishizaki *et al.*, 1990; Morse *et al.*, 1993). Therefore, the induction of NDMA-dI activity could lead to an increased production of reactive alkylating species that could attack DNA and other macromolecules, either in the liver, or bladder of *S. mansoni*-infected animals. In support of this, it has been found that the level of O^6-methyl-deoxyguanosine detected in the liver DNA of infected mice was directly proportional to the intensity of *S. mansoni* infection (Badawi *et al.*, 1993). Induction of NDMA-dI activity could be attributed, at least in part, to the stress incurred as a result of early hepatic inflammation (Thompson *et al.*, 1982). Alternatively, it may be due to the presence of *N*-nitrosamines, which are known to induce total CYP, and NDMA-dI activity (Sheweita & Mostafa, 1996).

It is known that CYP 1A1 participates in the bio-activation of PAHs and this CYP isozyme is believed to play an important role in human carcinogenesis (Guengerich, 1991). The carcinogenic potency of B(*a*)P and the extent of binding of its ultimate metabolites to proteins and DNA *in vitro* has been correlated with the induction of AHH activity and CYP 1A1 content (Kim *et al.*, 1992). CYP 1A1 protein could not be detected at 45 days p.i. and, at the same time, the activity of ethoxyresorufin *O*-deethylase was decreased by 97% relative to control value (fig. 1A, table 1) at this time. Furthermore, when B(*a*)P was activated by liver microsomes from *S. mansoni*-infected mice, the total binding of B(*a*)P metabolites to DNA also decreased at 45 days p.i. although at the early stages of infection (up to 30 days), the total binding of B(*a*)P metabolites to DNA was significantly increased, reaching a maximum at 15 days p.i. This agrees with our previous results where we found that both the total CYP content and the activity of AHH were induced during the earlier stages of *S. mansoni* infection (Sheweita & Mostafa, 1995; Sheweita *et al.*, 1998). Therefore, the genotoxic effects resulting from B(*a*)P metabolism could increase liver damage at the early stages of infection. In support of this, it has been found that the incidence of 2-AAF induced liver tumours is significantly higher in *S. japonicum*- or *S. mansoni*-infected mice (Miyasato, 1984; Kakizoe, 1985). Furthermore, the liver tumours resulting from schistosome infections were more frequent, developed earlier and were more advanced than those in non-infected mice. In addition, the metabolic activation of carcinogens was enhanced in *S. haematobium*-infected hamsters, suggesting that an alteration in host metabolism following schistosome infection might be an important factor in carcinogenesis. It is now clear that schistosomiasis enhances and accelerates the carcinogenic effects of some pro-carcinogens such as B(*a*)P and 2-AAF (Miyasato, 1984; Kakizoe, 1985; Ishii *et al.*, 1994).

Humans are exposed to certain coumarins in the diet. Most coumarin compounds are bio-activated by CYP 1A1 and, to a lesser extent by other CYP isozymes, to reactive intermediates that subsequently form covalent linkages with the apoprotein and induce different types of toxicity and carcinogenicity in both humans and rats (Lake *et al.*, 1989; Lake, 1999; Zhuo *et al.*, 1999). In the present study, the activity of ethoxycoumarin hydroxylase was found to decrease following *S. mansoni* infection, reaching 40% of the control value at 45 days p.i. (table 3). This correlates well with the decrease in CYP 1A1 activity observed over the same period (fig. 1A). The expression of other CYP isozymes, including 2C6, 2B1/2 and 4A was also followed, since there is evidence that these CYP isozymes are also involved in

Table 3. The effect of *Schistosoma mansoni* infection on the benzo(*a*)pyrene-DNA binding capacity of mouse liver microsomes.

	Benzo(*a*)pyrene-DNA adducts (RI/100 μg DNA)[a]	
Days post-infection	Control	Infected
6	4.7±0.69	8.0±0.66 (+70%, $P<0.01$)
15	4.7±0.69	12.3±1.2 (+161%, $P<0.001$)
30	4.7±0.69	8.8±0.59 (+87%, $P<0.001$)
45	4.7±0.69	1.3±0.42 (−72%, $P<0.005$)

[a] Means±SE of six mice.

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the bio-activation of toxins and carcinogens (Allis *et al.*, 1996; Hanioka *et al.*, 1996). Thus, the expression of CYP 2C6 was induced at 6 and 15 days but decreased compared to controls at 45 days p.i. (fig. 1C). Interestingly, CYP 2B1/2 protein expression remained similar to the controls at all time points with the exception of 45 days post infection, when it could not be detected by the Western blot assay (fig. 1B). Evidence that this was indeed a real phenomenon was obtained by assaying the activity of pentoxyresorufin *O*-dependentylase, an enzyme marker for CYP 2B1/2, which also showed a dramatic 96% decrease in activity at 45 days p.i. (table 2).

In conclusion, the present study demonstrates alterations in the cellular levels of CYP isozymes that are responsible for the bio-activation of various carcinogens and xenobiotics. Our results show that the expression of all tested CYP isozymes decreased over the same period, especially CYP 1A1 and CYP 2B1/2, which could not be detected by Western blot analysis, nor by specific enzyme assays at 45 days p.i. The non-expression of CYP 1A1 during the later stages of infection could prolong the exposure of liver and other organs to PAHs and other toxic compounds without detoxification or activation. Moreover, *S. mansoni* infections may change the intensity and the pharmacological actions of many drugs, e.g. anti-schistosomal drugs, which are also mainly metabolized by the P450 system in the liver of infected individuals. Such changes should be considered when xenobiotics are administered to patients with schistosomiasis.

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References

Allis, J.W., Brown, B.L., Simmons, J.E., Hatch, G.E., McDonald, A. & House, D.E. (1996) Methanol potentiation of carbon tetrachloride hepatotoxicity: the central role of cytochrome P450. *Toxicology* **16**, 131–140.

Al-Shukri, S., Alwan, M.H., Nayef, L.H. & Rahman, A.A. (1987) Bilharziasis in malignant tumors of the urinary bladder. *British Journal of Urology* **59**, 59–62.

Arcos, J., Davies, D.L., Brown, C.E. & Argus, M.E. (1977) Repressible and inducible enzymatic forms of DMN-demethylase. *Zeitschrift für Krebsforschung und Klinische Onkologie* **89**, 181–199.

Ashurst, S.T. & Cohen, G.L. (1981) The formation and persistence of benzo(*a*)pyrene metabolite-deoxyribonucleoside adducts in rat skin *in vivo*. *International Journal of Cancer* **28**, 387–392.

Badawi, A.F., Cooper, D.P., Mostafa, M.H., Doenhoff, M.J., Probert, A., Fallon, P., Cooper, R. & O'Connor, P.J. (1993) Promutagenic methylation damage in liver DNA of mice infected with *Schistosoma mansoni*. *Carcinogenesis* **14**, 653–657.

Bartsch, H. & Montesano, R. (1984) Relevance of nitrosamines to human cancer. *Carcinogenesis* **5**, 1381–1393.

Bjelogrlic, N., Peng, R., Park, S.S., Gelboin, H.V., Honkakoski, P., Pelkonen, O. & Vahakangas, K. (1993) Involvement of P4501A1 in benzo(*a*)pyrene but not in benzo(*a*)pyrene-7,8-dihydrodiol activation by 3-methylcholanthrene-induced mouse liver microsomes. *Pharmacology and Toxicology* **73**, 319–324.

Burke, M.D., Thompson, S., Elcombe, C.R., Halpert, J., Haaparanta, T. & Mayer, R.T. (1985) Ethoxy-, pentoxy- and benzyloxyphenoxazones and homologues: a series of substrates to distinguish between different induced cytochromes P450. *Biochemical Pharmacology* **34**, 3337–3345.

Butler, M.A., Iwasaki, M., Guengerich, F.P. & Kadlubar, F.F. (1989) Human cytochrome P450 (P450 1A2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proceeding of the National Academy of Sciences, USA* **86**, 7696–7700.

Camus, A.M., Geneste, O., Honkakoski, P., Bereziat, J.C., Henderson, C.J., Wollf, C.R., Bartsch, H. & Lang, M.A. (1993) High variability of metabolism among individuals: role of cytochrome P450 2A6 and 2E1 in the dealkylation of *N*-nitrosodimethylamine and *N*-nitrosodiethylamine in mice and humans. *Molecular Carcinogenesis* **7**, 268–275.

Cheever, A.W. (1978) Schistosomiasis and neoplasm. *Journal of the National Cancer Institute* **61**, 13–18.

Conney, A.H. (1982) Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons. *Cancer Research* **42**, 4875–4917.

Cooper, D.P., Yoa, G.F., Qu, Y.H. & O'Connor, P.J. (1991) DNA methylation in individuals at high risk for stomach cancer. *British Journal of Cancer* **13**, 65–71.

Delmas, V., Daug, M.C., Davody, A.P., Coulaud, J.P. & Moulonguet, A. (1986) Carcinoma of the bladder in urinary schistosomiasis. *Annals of Urology* **20**, 213–217.

El-Mouelhi, M., Black, M. & Phillips, M. (1987) Hepatic cyctochrome P450 system in experimental hepatosplenic schistosomiasis. Presence of an artifact in spectrophotometric analysis. *Biochemical Pharmacology* **36**, 2621–2626.

Everson, R.B., Randerath, E., Santella, R.M., Cefalo, R.C., Avitts, T.A. & Randerath, K. (1986) Detection of smoking-related covalent DNA adducts in human placenta. *Science* **231**, 54–57.

Gelboin, H.V. (1980) Benzo(*a*)pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiological Reviews* **60**, 1107–1166.

Gonzalez, F.J. & Gelboin, H.V. (1994) Role of human cytochrome P-450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metabolism Reviews* **26**, 165–171.

Gooderham, N.J. & Mannering, G.J. (1985) Depression of the hepatic cytochrome P450 monooxygenase system by treatment of mice with the anti-neoplastic agent, 5-azacytidine. *Cancer Research* **45**, 1569–1572.

Greenle, W.F. & Poland, A. (1978) An improved assay of 7-ethoxycoumarin O-deethylase activity: induction of hepatic enzyme activity in C57BL/6J and DBA/2J mice by phenobarbital, 3-methylcholantherene and 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Journal of Pharmacology and Experimental Therapeutics* **205**, 596–605.

Guengerich, F.P. (1991) Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chemical Research Toxicology* **4**, 391–407.

Habib, S.L., Sheweita, S.A., Awad, A., Mashaal, N., Soliman, A. & Mostafa, M.H. (1996) Influence of *Schistosoma mansoni* infection on carcinogen-metabolizing capacities and *in vitro* aflatoxin B_1 metabolism in human liver. *Oncology Reports* **3**, 769–773.

Hammons, G.J., Guengerich, F.P., Weis, C.C., Beland, F.A. & Kadlubar, F.F. (1985) Metabolic oxidation of carcinogenic arylamines by rat, dog and human hepatic microsomes and by purified flavin-containing and cytochrome P450 monooxygenases. *Cancer Research* **45**, 3578–3585.

Hanioka, N., Omae, E., Nishimura, T., Jinno, H., Onodera, S., Yoda, R. & Ando, M. (1996) Interaction of 2,4,4′-trichloro-2′-hydroxydiphenyl ether with microsomal cytochrome P450-dependent monooxygenases in rat liver. *Chemosphere* **33**, 265–276.

Harris, C.C., Vahakangas, K., Newman, M.J., Trivers, G.E., Shamsuddin, A.K., Sinopoli, N.T., Mann, D.L. & Wright, W.E. (1985) Detection of benzo(a)pyrene diolepoxide in serum from coke oven workers. *Proceedings of the National Academy of Sciences, USA* **82**, 6672–6676.

Hashem, M. & Boutros, K. (1961) The influence of bilharzial infection on the carcinogenesis of the bladder. An experimental study. *Journal of the Egyptian Medical Association* **44**, 598–606.

Hasler, J.A., Siwela, A.H., Nyathi, C.B. & Chetsanga, C.J. (1986) The effect of schistosomiasis on the activation of aflatoxin B_1. *Research Communications in Pathology and Pharmacology* **51**, 421–423.

Hill, M.J. (1988) *N*-nitroso compounds and human cancer. pp. 90–102 *in* Hill, M.J. (*Ed.*) *Nitrosamine toxicology and microbiology.* Chichester, Ellis Horwood.

Ishii, A., Matsuoka, H., Aji, T., Ohta, N., Arimoto, S., Wataya, Y. & Hayatsu, H. (1994) Parasite infection and cancer: with special emphasis on *Schistosoma japonicum* infection (Trematoda). A review. *Mutation Research* **305**, 273–281.

Ishizaki, H., Brady, J.F., Ning, S.M. & Yang, C.S. (1990) Effect of phenethyl isothiocyanate on microsomal N-nitrosodimethylamine metabolism and other monooxygenase activities. *Xenobiotica* **3**, 255–264.

Jerina, D.M., Yagi, H., Thakker, D.R., Karley, J.M., Mak, H.D., Boyd, D.R., Gadaginamath, G., Wood, A.W., Buening, M., Chang, R.L., Levin, W. & Conney, A.H. (1979) Stereoselective metabolic activation of polycyclic aromatic hydrocarbons. *Advanced Pharmacology and Therapeutics* **9**, 53–62.

Kakizoe, Y. (1985) The influence of *Schistosoma mansoni* infection on carcinogenesis of mouse livers initiated by N-2-fluorenylacetamide. *Kurume Medical Journal* **32**, 169–178.

Kim, S.Y., Chung, J.H., Kang, K.W., Joe, C.O. & Park, K.H. (1992) Relationship between activities of cytochrome P450 monooxygenases in human placental microsomes and binding of benzo(a)pyrene metabolites to calf thymus DNA. *Drug Chemistry and Toxicology* **15**, 313–327.

Lake, B.G. (1999) Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. *Food Chemistry and Toxicology* **37**, 423–453.

Lake, B.G., Gray, T.J., Evans, J.G., Lewis, D.F., Beamand, J.A. & Hue, K.L. (1989) Studies on the mechanism of coumarin-induced toxicity in rat hepatocytes: comparison with dihydrocoumarin and other coumarin metabolites. *Toxicology and Applied Pharmacology* **97**, 311–323.

Lake, B.G., Charzat, C., Tredger, J.M., Renwick, A.B., Beamand, J.A. & Price, R.J. (1996) Induction of cytochrome P450 isoenzymes in cultured precision-cut rat and human liver slices. *Xenobiotica* **3**, 297–306.

Lijinsky, W., Conrad, E. & Van De Bogart, R. (1972) Nitrosoamines formed by drug/nitrite interactions. *Nature* **239**, 165–174.

Lowry, O.H., Rosbrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193**, 265–275.

Manchester, D.K., Bowman, E.D., Parker, N.B., Caporaso, N.E. & Weston, A. (1992) Determinants of polycyclic aromatic hydrocarbons-DNA adducts in human placenta. *Cancer Research* **52**, 1499–1503.

Mclean, A.E. & Day, P.A. (1974) The use of new methods to measure the effect of diet and inducers of microsomal enzyme synthesis on cytochrome P-450 in liver homogenates, and on metabolism of dimethylnitrosamine. *Biochemical Pharmacology* **23**, 1173–1180.

Miyasato, M. (1984) Experimental study of the influence of *Schistosoma japonicum* infection on carcinogenesis of mouse liver treated with N-2-fluorenylacetamide (2-FAA). *Japanese Journal of Parasitology* **33**, 41–48.

Morse, M.A., Zu, H., Galati, A.J., Schmidt, C.J. & Stoner, G.D. (1993) Dose-related inhibition by dietary phenethyl isothiocyanate of esophageal tumorigenesis and DNA methylation induced by N-nitrosomethylbenzylamine in rats. *Cancer Letters* **72**, 103–110.

Mostafa, M.H. & Sheweita, S.A. (1992) Modification of the oxidative *N*-demethylation of dimethylnitrosamine by various anti-inflammatory drugs. *Ramazzini Newsletters* **2**, 15–22.

Nash, T. (1953) The calorimetric estimation of formaldehyde by means of Hantzsch reactions. *Biochemical Journal* **55**, 416–421.

Pohl, R.J. & Fouts, J.R. (1980) A rapid and sensitive method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Analytical Biochemistry* **107**, 150–155.

Preussmann, P. (1984) Occurrence and exposure to *N*-nitroso compounds and precursors. pp. 3–15 *in* O'Neil, I.K., Von Borstll, R.C., Miller, C.T., Long, J. & Bartsch, H. (*Eds*) *N-nitroso compounds: occurrence, biological effects and relevance to human cancer.* International Agency for Research on Cancer, IARC Sci, Lyon.

Savela, K. & Hemminki, K. (1991) DNA adducts in lymphocytes and granulocytes of smokers and nonsmokers detected by the ^{32}P-postlabeling assay. *Carcinogenesis* **12**, 503–508.

Sheweita, S.A. (2000) Drug-metabolizing enzymes: mechanisms and functions. *Current Drug Metabolism* **1**, 107–132.

Sheweita, S.A. & Mostafa, M.H. (1995) Recovery of the hepatic carcinogen-metabolizing capacity in schistosome-infected mice after treatment with the

antischistosomal praziquantel. *Oncology Reports* **2**, 155–159.

Sheweita, S.A. & Mostafa, M.H. (1996) *N*-nitroso compounds induce changes in carcinogen-metabolizing enzymes. *Cancer Letters* **106**, 243–249.

Sheweita, S.A., Habib, S.L. & Mostafa, M.H. (1997) Schistosomiasis induced changes in glutathione levels and glutathione reductase/glutathione S-transferase activities in human liver. *Biomedical Letters* **56**, 119–127.

Sheweita, S.A., Mangoura, S.A. & El-Shemi, A.G. (1998) Different levels of *Schistosoma mansoni* induce changes in drug-metabolizing enzymes. *Journal of Helminthology* **72**, 71–77.

Siwela, A.H., Nyathi, C.B., Chetsanga, C.J. & Hasler, J.A. (1990) The effect of schistosomiasis on the covalent binding of 2-acetylaminofluorene to mouse liver macromolecules *in vivo* and *in vitro*. *Biochemical Pharmacology* **40**, 379–382.

Smithers, S.R. & Terry, R.J. (1965) The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. *Parasitology* **55**, 695–700.

Thompson, T., Watkins, J. & Gergus, Z. (1982) Effect of microsomal enzyme inducers on the soluble enzymes of phase II biotransformation. *Toxicology and Applied Pharmacology* **66**, 400–408.

Umbenhauer, D., Wild, C.P., Montesano, R., Saffhill, R., Boyle, J.M., Huh, N., Kirstein, U., Thomale, J., Rajewski, M.F. & Lu, S.H. (1985) O^6-Methyldeoxyguanosine in oesophageal DNA among individuals at high risk of oesophageal cancer. *International Journal of Cancer* **36**, 661–665.

Vahakangas, K., Raunio, H., Pasanen, M., Sivonen, P., Park, S.-S., Gelboin, H.V. & Pelkonen, O. (1989) Comparison of the formation of benzo(a)pyrene diolepoxide-DNA adducts *in vitro* by rat and human microsomes: evidence for the involvement of P450 1A1 and P450 1A2. *Journal of Biochemical Toxicology* **4**, 79–83.

Venkatesan, N., Arcos, J.C. & Argus, M.F. (1968) Differential effect of polycyclic hydrocarbons on the demethylation of the carcinogen dimethylnitrosamine by rat tissues. *Life Science* **7**, 1111–1118.

Wattenberg, L.W. (1987) Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo(a)pyrene on pulmonary and forestomach neoplasia in A/J mice. *Carcinogenesis* **12**, 1971–1973.

World Health Organization (1985) The control of schistosomiasis. Report of WHO Expert Committee. Technical Report Series No. 728, WHO, Geneva.

World Health Organization (1986) The epidemiological association between *Schistosoma haematobium* infection and bladder cancer. Report of WHO Expert Committee. Technical Report Series WHO/Schist/86. WHO/CAN/86.1, Geneva.

Yang, C.S., Yoo, J.S., Ishizaki, H. & Hong, J.Y. (1990) Cytochrome P450IIE1: roles in nitrosamine metabolism and mechanisms of regulation. *Drug Metabolism Reviews* **22**(2–3), 147–159.

Yoo, J.S., Ishizaki, H. & Yang, C.S. (1990) Roles of cytochrome P450IIE1 in the dealkylation and denitrosation of N-nitrosodimethylamine and N-nitrosodiethylamine in rat liver microsomes. *Carcinogenesis* **12**, 2239–2243.

Yoo, J.S.H., Guengerich, F.P. & Yang, C.S. (1988) Metabolism of *N*-nitrosodialkylamines by human liver microsomes. *Cancer Research* **88**, 1499–1504.

Zhuo, X., Gu, J., Zhang, Q.Y., Spink, D.C., Kaminsky, L.S. & Ding, X. (1999) Biotransformation of coumarin by rodent and human cytochromes P-450: metabolic basis of tissue-selective toxicity in olfactory mucosa of rats and mice. *Journal of Pharmacology and Experimental Therapeutics* **288**, 463–471.

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